Nanotechnology in the Life Sciences

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Green Nanoparticles

Synthesis and Biomedical Applications

Springer

Nanotechnology in the Life Sciences

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Nano and biotechnology are two of the 21st century's most promising technologies. Nanotechnology is demarcated as the design, development, and application of materials and devices whose least functional make up is on a nanometer scale (1 to 100 nm). Meanwhile, biotechnology deals with metabolic and other physiological developments of biological subjects including microorganisms. These microbial processes have opened up new opportunities to explore novel applications, for example, the biosynthesis of metal nanomaterials, with the implication that these two technologies (i.e., thus nanobiotechnology) can play a vital role in developing and executing many valuable tools in the study of life. Nanotechnology is very diverse, ranging from extensions of conventional device physics to completely new approaches based upon molecular self-assembly, from developing new materials with dimensions on the nanoscale, to investigating whether we can directly control matters on/in the atomic scale level. This idea entails its application to diverse fields of science such as plant biology, organic chemistry, agriculture, the food industry, and more.

Nanobiotechnology offers a wide range of uses in medicine, agriculture, and the environment. Many diseases that do not have cures today may be cured by nanotechnology in the future. Use of nanotechnology in medical therapeutics needs adequate evaluation of its risk and safety factors. Scientists who are against the use of nanotechnology also agree that advancement in nanotechnology should continue because this field promises great benefits, but testing should be carried out to ensure its safety in people. It is possible that nanomedicine in the future will play a crucial role in the treatment of human and plant diseases, and also in the enhancement of normal human physiology and plant systems, respectively. If everything proceeds as expected, nanobiotechnology will, one day, become an inevitable part of our everyday life and will help save many lives.

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Preface

Advances in nanotechnology and engineering have revolutionized the materials used in our daily lives, and on which our societies and economies are based. While various nano-based products are increasingly used in different economic sectors, there is an increased awareness regarding the environmental and biological safety related to their preparation and uses. Green nanotechnology is certainly the most important division of nanotechnology, which uses 12 principles of green chemistry to promote sustainability and minimize health risks. This book, *Green Nanoparticles: Synthesis and Biomedical Applications*, outlines how green nanotechnology has been used to produce more efficient, reliable, and eco-friendly products and devices for biomedical, food, and agricultural applications. This volume certainly represents an important source of information for scientists who want to learn more about the current status and future perspectives of the use of green nanotechnology to create the next generation of products which could solve current and future challenges faced by the biomedical, food, and agricultural fields, as well society in general. The book contains 20 chapters covering topics related to the application of nanotechnology in the development of topical delivery systems, stimuli-responsive nanocarriers, biosensors, and the treatment of neglected tropical diseases. It also describes the uses of plants to produce green nanoparticles and evaluates the potential toxicity of nanomaterials. In this way, we believe that this book can provide knowledge to different sectors such as academia, industry, stakeholders, and anyone who has an interest in the improvements of green nanotechnology.

We are indebted to the authors who contributed in this book. We wish to thank Dr. Emmy Lee, Associate Editor, Springer Nature Korea Limited, for her generous assistance and persistence in finalizing the edited volume. Special thanks are due to our valued fellow colleagues and university authorities for their kind support and continuous inspiration throughout the task.

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Chapter 1
Biomedical Applications of Stimuli-Responsive Hydrogels

Anderson Ferreira Sepulveda, Roger Borges, Juliana Marchi, and Daniele Ribeiro de Araujo

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1 Introduction

A copolymer is a macromolecule derived from more than one species of polymers, consisting of two or more blocks of different polymers chemically bonded to one another. Among the considerable number of copolymer types, one of the most used is polyethylene glycol (PEG)-based polymers, including their blocks (with two or

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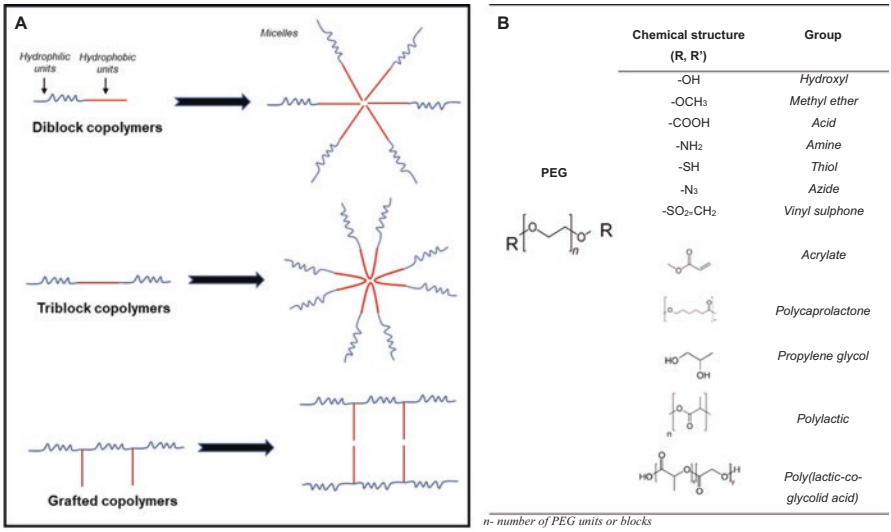

Fig. 1.1 Schematic representation of diblock, triblock, grafted copolymers, and their self-assembly in polymeric micelles (diblock and triblock) and their aggregates (grafted) (**a**). Polyethylene glycol (PEG) structure and some chemical groups used as its functional radicals on R and/or R' positions (**b**)

three and graft copolymers) (IUPAC 1997). PEG is a relatively hydrophilic and linear polymer, synthetized by polymerization of ethylene oxide units resulting in molecular weights ranging from 0.4 to 100 kDa and arrangements, such as micelles, according to the conjugation with hydrophobic polymers.

The presence of hydroxyl end groups allows the formation of covalent bindings with a variety of chemical groups (polylactic acid, poly(amino acid), polycaprolactone, poly(lactic-co-glycolic) acid, acrylate, acetylene, etc.) (Fig. 1.1), resulting on particular physicochemical features and different self-assembly mechanisms and biological properties, such as interaction with proteins and peptides as well as the formation of biocompatible hydrogels matrices for drug delivery, tissue regeneration, and diagnosis platforms (Zhu et al. 2010; Boonlai et al. 2018; Qureshi et al. 2019). In fact, the association of hydrophobic and hydrophilic groups into the PEG-based chemical structure evokes the formation of self-assembled aggregates such as micelles and/or hydrogels, in response to concentration and environmental conditions such as temperature, UV light, pH, and ionic strength.

Hydrogels are defined as polymeric networks capable to absorb a significant water content, forming highly permeable matrices with several biomedical applications including small molecules and proteins carriers and scaffolds for cells growing and tissue regeneration. In fact, the main property of those materials is their capability for presenting as highly viscous materials with gradual dissolution after application associated with their ability for responding upon certain environmental conditions (such as temperature and pH), according to the polymeric composition

and concentration (Zhang et al. 2014; Moeinzadeh and Jabbari 2015; Kong et al. 2017; Chen et al. 2019).

In this context, PEG-based hydrogels have been used for several biomedical purposes considering their biocompatible, non-immunogenic, high purity, adequate physicochemical stability, and rheological properties. In special, this chapter focuses on PEG-based temperature and pH-sensitive hydrogels presenting their composition, mechanical properties, supramolecular structure, and self-assembled mechanisms, as well as highlighting their progress as biocompatible matrices for biomedical applications.

2 PEG-Based Temperature-Sensitive Hydrogels: Structural and Physicochemical Properties

Among temperature-sensitive materials, PEG-based polymers are considered one of the most used materials, especially for biomedical applications, which allows the incorporation of cells, drugs, and other biocompatible polymers into their porous matrix. In fact, the ability to form hydrogels is observed when this material is delivered in solution and, in response to the physiological temperature, their viscosity is instantaneously changed, reaching the sol-gel transition temperature and adequate mechanical properties for in situ depot systems. The main vantage attributed to those systems is that there is no need of chemical agents for the hydrogels formation, which results in materials with low biological toxicity (Klouda 2015). For this reason, these hydrogels have been pointed as one of the main matrices for delivery of drugs and other bioactive molecules. Also, the conjugation of PLGA and PCL with PEG have gained attention because of their biodegradability and biocompatibility. On the other hand, other polymer types, such as PEG-poly-N-isopropylacrylamide (PNIPAAm), produce hydrogels by chemical cross-link, and the chemical initiator must be removed from the systems for reducing the formulation toxicity. In this sense, physical methods are preferred for hydrogels formation, resulting on the formation of gels with adequate mechanical strength and capability to modulate the drug release rate for a long period of time (Alexander et al. 2014).

One of the most accepted mechanisms for explaining the thermogellification phenomenon concerns the interaction between the copolymer units. The monomers of these copolymers, at concentrations above the critical micellar concentration (CMC), are organized in micelles in aqueous medium in order to minimize free energy. As the temperature increases, the equilibrium between micelles and unimers is favored in the direction of micellization due to the dehydration of the hydrophobic units, conferring to the system new structural organization, observed by the formation of polymeric networks. As an example, copolymers composed of PEG-PPG-PEG are self-assembled in micelles, at the critical micellar concentration (CMC), and then organized as different supramolecular structures that assume lamellar, hexagonal, and cubic and the coexistence among them (Oshiro et al. 2014;

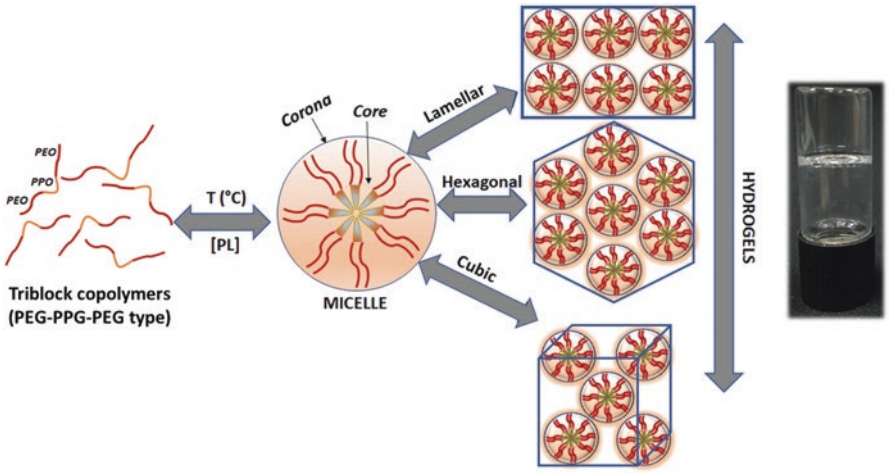

Fig. 1.2 Representation of triblock copolymers (e.g., PEG-PPG-PEG) thermoreversible self-assembly in micelles and hydrogels. In detail, the hydrogels supramolecular structures (lamellar, hexagonal, and cubic) are shown. Polyethylene glycol (PEG), propylene glycol (PPG)

Nascimento et al. 2018; Mariano et al. 2019) (Fig. 1.2). This phenomenon is reversible and below the sol-gel transition temperature (Tsol-gel), the hydrophobic chains are rehydrated, and the micelles restructured in solution (Jeong et al. 2002; Ur-Rehman et al. 2010).

Several reports have studied the thermogelation mechanisms, using a sort of techniques for characterizing the hydrogels. In special, the main employed techniques are rheology and small-angle X-ray scattering (SAXS) for determining the sol-gel transition temperature, mechanical properties, and the phase organization behavior, establishing relationships among composition, architecture, and hydrogels biological performances. Some of those studies are discussed throughout the next section.

2.1 Hydrogels Mechanical Properties and Phase Organization Studied by Rheological Analysis and Small-Angle X-Ray Scattering (SAXS): Implications on Drug-Controlled Release

Rheological studies and mechanical properties characterization are important tools to analyze hydrogel behavior, as elastic and viscous materials. It allows interpreting microscopic or internal structural changes under gradual heating or continuous shearing. The oscillatory (or dynamic) experiments are accomplished to study rheological behavior, where a sinusoidal shear is applied to the sample. Linear visco-

elastic properties can be determined by time-dependent response of soft matter in small-amplitude oscillatory shear experiments (Barbucci et al. 2002).

Elastic behavior is the material ability to restore its original shape when the external force is removed, normally referred to as elastic modulus, also known as storage modulus (G'). The viscous modulus (or shear loss modulus G") is a property that shows how any deformation ceases when there is no more an external force. In general, thermosensitive hydrogels are viscoelastic materials, which display a temperature- and concentration-dependent phase angle.

In general, hydrogels can be more elastic (non-Newtonian or Hookean material) or viscous liquid (Newtonian material), depending on value G"/G' ratio. These properties are related by the systems molecular configuration. Under the micellization temperature, unimers are dispersed in solution, which confers a sol phase. At micellization temperature, unimers begin to self-assemble in isolated micelle structures, but the system continues in sol-gel state with low G' and G" values until they reach the sol-gel transition temperature (Tsol-gel). At Tsol-gel, it starts to agglutinate micelles in more complex structures, increasing G' values, and the material is then converted in a gel state with a G' > G". Furthermore, the copolymer structure is affected by the degree of entanglement, and, if this structure is symmetric, it ensues the maximum elasticity and viscosity (Lee et al. 2009) by higher degree of solvation of micelle shell than unimers chains (Prud'homme et al. 1996).

For thermosensitive materials, the viscosity in sol phase declines slowly on warming. This is ascribed to an increase in micellization and lowered solvent viscosity. However, it is observed a steep increase in viscosity when it is near to sol-gel point transition, what can be attributed to the reduction of intermicellar space and micelle entanglements (Yokaichiya et al. 2017). As it has been seen, the rheological properties of a thermosensitive hydrogel are controlled by the micellar phase organization that is studied by small-angle scattering techniques.

Small-angle scattering (SAS) techniques characterize macromolecular structures and dimensions by the incidence of X-ray or neutron beams on electronic cloud (SAXS) or atomic nucleus (SANS), respectively (Svergun, 2010; Jacques and Trewhella 2010). These beams generate coherent secondary waves after suffering interference. Resulting waves can be destructive or constructive, which allows the formation of diffraction patterns, usually described in the form of intensity I as a function of scattering vector amplitude q:

$$q = \frac{4\pi \sin\theta}{\lambda} \tag{1.1}$$

where λ is the wavelength of incident radiation and 2θ is the angle between incident and reflected beam. SAS techniques are known as low-resolution ones because it is not possible to determine atomic coordinates (like X-ray crystallography), just the shape and size of analyzed structure.

SAXS and SANS allow the observation of the supramolecular structure and the interactions between the different functional chemical groups, since wavelengths on

the nanometer scale of X-rays and neutron beams allow the observation of interatomic interactions (Putnam et al. 2007; Imae et al. 2011). SAS techniques are ideal to study soft gel materials, since it does not require crystal form and elaborated preparations, like X-ray crystallography. Therefore, they are being used to clarify different hydrogel structures. It has been pointed out that the organization of micellar aggregates in the cubic phase exhibits gel properties; however, depending on copolymers concentrations, hexagonal and lamellar phases may be formed, although they are characteristic of anisotropic molecular ordering (Chaibundit et al. 2007; Newby et al. 2009; Ulrich et al. 2012; Basak and Bandyopadhyay 2013; Nascimento et al. 2018; Mariano et al. 2019).

Rheological alterations are also related to micellar supramolecular organization. A possible mechanism for the micellar rearrangement to occur is "hard sphere crystallization" under cubic, hexagonal, or lamellar phase, due to packing of spherical micelles, what can be evidenced by small-angle X-ray scattering (SAXS) or small-angle neutron scattering (SANS) experiments and identification of Bragg diffraction peaks (Prud'homme et al. 1996; Artzner et al. 2007; Oshiro et al. 2014). However, it is well known the concentration and temperature role on phase organization type for each copolymer type. SANS results point that micelle shells are overlapped as enhancing PL concentration. Using SANS technique is possible to follow structural transition of hydrogels, suggesting that micelles initiate to agglutinate when PEG monomers (which are in micellar corona) become more hydrophobic and break hydrogen bonds with water following the temperature increased.

Hydrogels are thixotropic materials, showing a reversible transition on their structure because of viscosity alteration induced by temperature and/or pH changes. This property is important to define the therapeutic efficacy of the hydrogel formulations to pharmaceutical purposes, due to their ability to extend retention time at the application site and to enhance the systemic bioavailability of some drugs (Ricci et al. 2005; Lee et al. 2009; Akkari et al. 2016). Sol-gel systems, which have non-Newtonian behavior, present yield values that are required to break down the semi-solid structure and to initiate the plastic flow. Then, since these yield values are increased, it is possible to indicate a gradual strengthening of the three-dimensional network structure.

The body fluid elements, mainly water, are the major factors in controlling the yield value, altering the systems structure. These elements can be diffused into the hydrogel matrix, affecting the number of cross-links formed and the hydration level. It has been demonstrated that enhancing cross-links and reducing the hydration level is possible to change the release rate of the encapsulated drugs. As viscous matrices, hydrogels are barriers to the drug release, since high viscosity hydrogels with swelled micelles tend to retain incorporated molecules for a long period of time. Thus, the PEG-based copolymer type, its molecular weight, and the addition of high-viscosity polymers into PEG-based hydrogels, forming hybrid systems with high molecular weight natural polymers (such as cellulose derivatives, hyaluronic acid), for example, can be differential factors to change materials properties for a specific biomedical purpose.

2.2 *Biomedical Applications of Thermosensitive PEG-Based Hydrogels: From Structural Organization to Biopharmaceutical Use*

Several in situ PEG-based hydrogels have been synthetized considering the insertion of biodegradable polyesters, showing to be good matrices for drug delivery systems and tissue repair. A possible disadvantage of these systems is the incorporation of thermolabile drugs, since the hydrogels preparation must be performed at low temperatures. On the other hand, PEG conjugation with high crystallinity and hydrophobicity polymers, such as PLGA and PCL, increases the drug incorporation percentage and changes the hydrogels structural organization, their morphology, and degradation rate (Deng et al. 2019). Regarding the biocompatibility, other PEG-based copolymers, PEG-PPG-PEG-based copolymers (such as poloxamers and poloxamines), different safety studies in clinical practice have reported their approval by FDA and use as pharmaceutical excipients (Cho et al. 2012). In this section, it will be discussed the influence of structural and composition parameters on PEG-derivatives hydrogels biomedical applications and implications when associated with other biodegradable polymers (hyaluronic acid, poly(N-(2-hydroxypropyl)) methacrylamide mono-/dilactate) and/or forming hybrid systems with laponite, gold nanoparticles, and liposomes among other nanocarriers. Although the formulation and physicochemical characterization of PEG-based hydrogels have been reported by several studies, the relationships between chemical modifications on PEG molecule and its biomedical application have been discussed on few studies.

The synthesis of PEG-PCL-PEG hydrogels as delivery systems for timolol maleate was reported by Mishra et al. (2011). In this study, comparisons with PVA showed more pronounced sol-gel transition temperatures and low cytotoxic effects in rabbit corneal epithelial culture cells when compared to PVA. In fact, the gelation of PEG-PCL-PEG polymers is dependent on the length and molecular weight of the PCL units, since hydrophobic interactions are the main driving forces observed on reversible sol-gel transitions (Deng et al. 2019). In a similar study, PEG-PCL-PEG hydrogels were reported as insulin delivery system. The rheological characterization was strictly related to the formulation injectability, since it was observed a Newtonian flow behavior with low viscosity (for 20% and 25% PEG-PCL-PEG) and a shear rate-dependent flow, as also observed for PEG-PPG-PEG hydrogels (such as poloxamer 407) (Payyappilly et al. 2014).

In attempt to observe the impact of structural parameters (molecular weight and the ratio of PEG-PCL blocks) on sol-gel transition, hydrogels formulations were also tested as scaffolds on highly porous surface for cell attachments obtaining promising results related to the maintenance of chondrocytes morphology, enhancing the cartilage regeneration, and providing a mechanically functional extracellular matrix (Deng et al. 2019). For other copolymer types, Alexander et al. (2014) described the preparation of PEG-PLGA-PEG-based hydrogels compared with PEG-PPG-PEG, regarding the dissolution rates, since PEG-PPG-PEG are promptly

removed from the site of injection, reducing their performance as in situ depot formulations related to PEG-PLGA-PEG. Other important advantage attributed to those polymers is the high PEG blocks biocompatibility, while PLGA blocks provide the molecule biodegradability due to the presence of ester links (Zentner et al. 2001).

The association between PEG and PLGA was also used as other triblock architectures, PLGA-PEG-PLGA. In special, a recent work showed the release of collagenase and trastuzumab controlled by those hydrogels looking forward antitumor efficacy in breast cancer (Pan et al. 2018). Also, the authors stated that the peritumoral administration is a potential strategy for the modulation collagen-rich extracellular matrix in solid tumors, provided by the enhancement of the interstitial transport after collagenase administration and the antibody efficacy. Other interesting result from this study was the comparison with clinical treatment regimens by the evaluation of the pre-formulation pharmacological effects in relation to hyaluronidase in combination to trastuzumab, since the hydrogel was able to trigger the intra-tumoral collagen degradation (Pan et al. 2018).

In this sense, the conjugation of cyclized succinyl ester groups into a PEG hydrogel matrix was proposed as bioadhesive medical sealant device for in vivo hemostasis, with the advantage of easy removal without causing tissue damage by mechanical debridement or surgical excision, enhancing the hemorrhagic control after administration in patients treated with anticoagulants (Bu et al. 2019). The use as implants was also investigated for infection prevention by Casadidio et al. (2018), when reported the development of hydrogels composed of vinylsulfonated triblock-PEG copolymers cross-linked with thiolated hyaluronic acid. The system was proposed for daptomycin local delivery in the management of implant-associated infection with the additional capability to reduce the drug chemical degradation, controlling the release rate and enhancing the in vitro antibiofilm activity against *S. aureus*. The association between vinylsulfonated triblock-PEG copolymers and thiolated hyaluronic acid was investigated as hydrogels for intra-articular injection in the management of osteoarthritis (Agas et al. 2019).

In other reports, hyaluronic acid was incorporated into poloxamer-based (PEG-PPG-PEG) hydrogels, organized as binary systems composed of poloxamer 407 and its more hydrophilic analog, poloxamer 338 aiming intra-articular therapy (Nascimento et al. 2018). The main observation from this study was the influence of hyaluronic acid on hydrogels phase organization, since SAXS patterns revealed transitions from lamellar to hexagonal phase and structural changes from cubic to gyroid and/or cubic to lamellar but maintaining the hydrogel-thermosensitive properties. Furthermore, the hybrid systems hyaluronic acid-PEG-PPG-PEG reduced in vitro cytotoxic effects, pointing their possible application as intra-articular drug delivery systems. In a similar report, the thermoreversible supramolecular assembly was observed for alpha-cyclodextrin incorporated to PEG-betulinic acid-hydroxycamptothecin, but the sol-gel transitions were determined by the length of PEG chains and the ratio between the drug-loaded micelles and alpha-cyclodextrin (Dai et al. 2017). This structural organization induced the sustained drug release,

enhanced the drug aqueous solubility, and showed appropriate micellar size for inducing a possible EPR effect.

Indeed, the development of PEG-based hybrid systems seems to be a tendency in the last years, since different reports discussed the formation of nanocomposites by associating laponite with PEG-PLGA diblock copolymers (Maeda et al. 2019). However, the insertion of laponite reduced the sol-gel transition temperature and caused a thermoresponsive concentration-dependent effect due to the adsorption of PEG-PLGA micelles on the laponite surface, as observed by SANS analysis. On the other hand, the incorporation of poly(allylamine)-grafted gold nanoparticles into PEG-PPG-PEG hydrogels did not show remarkable structural changes but demonstrated pronounced wound healing properties upon topical application for antibacterial activity (Mahmoud et al. 2019). Similar results were also obtained for hybrid systems composed of liposomal doxorubicin and PLGA-PEG-PLGA hydrogels (Cao et al. 2019), implying that the adequate rheological properties and viscosity allowed the use of this system for peritumor injection, which will improve the drug therapeutic effect and reduce its systemic toxicity.

Despite the promising biomedical applications as drug delivery systems or injectable cell scaffolds, PEG-PLGA-based triblock copolymers (or their derivative PLGA-PEG) show water solubility dependent on PLGA content compared to PEG, indicating an ideal PEG/PLGA ratio of 0.56 for obtaining a thermoresponsive hydrogel with appropriate aqueous solubility for injectable administration (Maeda et al. 2019). Other important feature is that during the synthesis process, molecular weight among cross-links should be studied by rheology in order to produce polymers with adequate elastic/viscous (G'/G") moduli relationships for gel or fluid formulations, since low viscosity at high shear rate is critical for painless injection (Payyappilly et al. 2014; Bu et al. 2019). In this context, some essential characteristics for adequate hydrogels biomedical performance and applications are summarized in Fig. 1.3.

2.3 pH-Sensitive PEG-Based Hydrogels: Theoretical Principles in pH-Sensitive Delivery Systems

Among the different chemical issues that influence a drug delivery system, the organ's pH arises as a critical property. For example, for a drug to come into the stomach, if it is taken orally, the drug should be able to withstand the alkaline salivary pH and then reach the stomach that, on the contrary, has an acidic pH. Most of the drugs are sensitive to pH and may lose their folding and therapeutic effect when in an inappropriate chemical environment. In this case, drug delivery systems are designed to hold the alkaline salivary pH and to deliver the drug into the stomach as it reaches an acidic pH (Liu et al. 2017). These systems are so-called as pH-sensitive or pH-responsive.

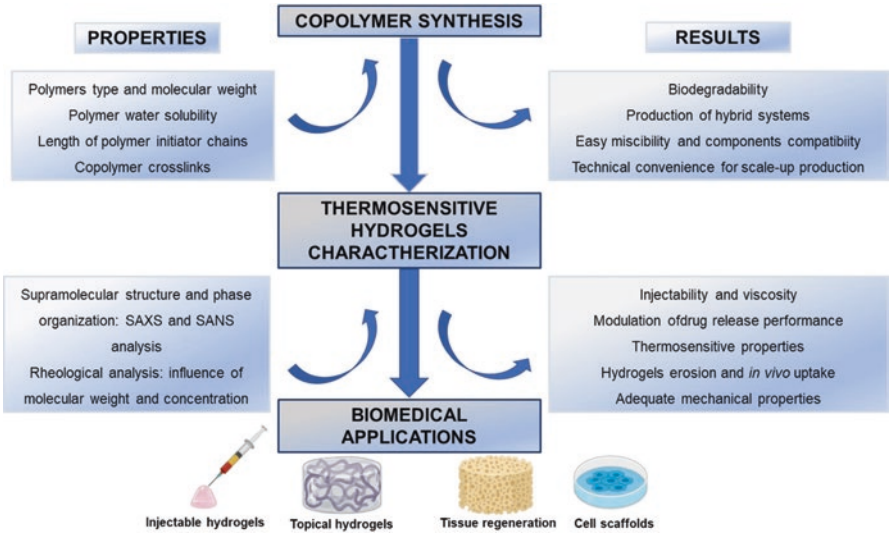

Fig. 1.3 Scheme of the main properties and results obtained for thermosensitive hydrogels synthesis and physicochemical characterization before their biomedical performance evaluation

In order to overcome the challenge of producing materials capable of resisting changes in the chemical environment in the human body, different pH-sensitive systems have been proposed in the literature (Liu et al. 2014, 2017). Currently, pH-sensitive drug delivery systems are designed by using inorganic, organic, or hybrid materials (a mixture of inorganic and organic materials) (Fig. 1.4).

Regarding inorganic materials, bioceramics like calcium phosphate (amorphous or crystalline hydroxyapatite) and zirconium oxide (ZrO_2) nanoparticles have been proposed as promising pH-sensitive carriers because of their high dissolution kinetics in acidic pH. It means that these ceramics can withstand alkaline pH, and delivery drugs at acidic pH, when they are degraded (Banerjee et al. 2011).

The class of pH-sensitive organic materials includes mostly polymers, liposomes, and micelles. If, on the one hand, inorganic materials become pH-sensitive because of their dissolution kinetic, organic materials, on the other hand, become pH-sensitive due to specific ionizable chemical groups found in their structure. Not all the organic molecules or polymers can become pH-sensitive, but as long as some ionizable chemical groups are grafted or functionalized into their structure, they can do so. Some of these ionizable chemical groups are carboxylic acids, amines, and phosphoric acids, among others. The fact that these chemical groups are ionizable means that they can be negatively or positively charged by donating or accepting protons, respectively (Shriver and Atkins 1999). Such property is related to their acid dissociation constant (Ka, which is more commonly referred to as pKa that, in turn, is its logarithmic representation), which consists of the equilibrium constant for a dissociation reaction in the context of an acid-base reaction (Shriver and Atkins 1999). When these organic molecules are either protonated or deprotonated due to

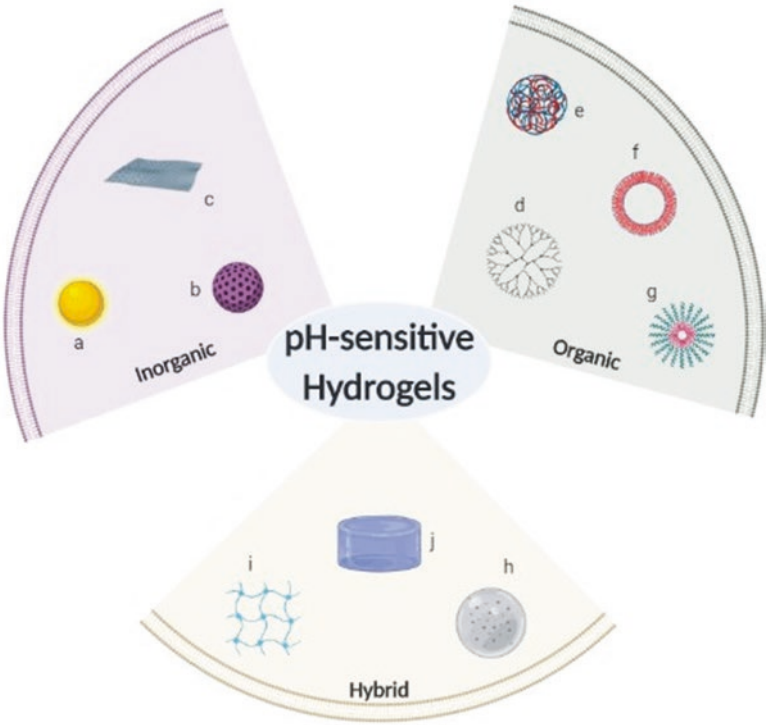

Fig. 1.4 The three main classes of materials used as pH-sensitive systems: inorganic (a, gold nanoparticles; b, zirconium oxide; c, hydroxyapatite delivery), organic (d, dendrimers; e, polymeric nanoparticles; f, liposomes; g, polymeric micelles), and hybrid systems (h, nanoparticles in hydrogel matrix; i, polymeric cross-linked hydrogels; j, micellar hydrogels)

their pKa and the pH of their environment, they can undergo three different conformational changes: (1) dissociation, (2) destabilization (by collapsing or swelling), and (3) changes in the partition coefficient between the vehicle and drug (Liu et al. 2014). Therefore, if these organic molecules are used to carry a drug into a specific site, they can deliver the drug when they suffer any of these conformational changes, releasing the drug into the desired environment.

Regarding polymeric materials, when such ionizable chemical groups are present in their structure, these polymers can either become cationic or anionic polymers. The names "cationic" or "anionic" polymers rely on the ability of the organic macromolecule to be ionizable at more acidic or basic pH, respectively. Also, there are some specificities about what type of ionizable chemical group can be found in these polymers. Usually, amino groups are used to produce cationic macromolecules, while carboxyl groups are used to produce anionic ones.

Cationic polymers with amino groups are more degradable in aqueous solution at acidic pH than in basic ones. As an illustration, aminoalkyl methacrylate copolymer (Eudragit E) is a Food and Drug Administration (FDA)-approved cationic

polymer having high solubility below pH 5. On the other hand, anionic polymers with carboxyl groups are more degradable in basic aqueous solution than in acidic pH. For example, poly(methacrylic acid-co-methyl methacrylate) (Eudragit L, S, and F), hydroxypropylmethylcellulose phthalate (HPMC-P), and HPMC acetate succinate (HPMC-AS) are conventional anionic polymers.

However, some polymers are neither cationic nor anionic, but it is not a limitation. Other strategies commonly employed to produce pH-sensitive polymers include functionalization and block copolymers. As long as there are innumerous polymers used in drug delivery applications, of course, the range of possibilities concerning pH-sensitive materials is extensive. Therefore, in the next sections, we shall keep our focus only on PEG-based pH-sensitive drug delivery systems. Despite all the almost infinite polymer candidate for pH-sensitive drug delivery systems, PEG has some advantages compared to other polymers, besides being an FDA-approved polymer and well-known behavior in the human body. Also, PEG is not an ionizable polymer, but some polymeric engineering techniques are used to make it pH-sensitive. We will explain why PEG is a proper polymer to be used in such delivery systems, as well as show what kind of applications are enabled when drug delivery systems based on PEG are employed.

2.4 Strategies to Make PEG pH-Sensitive: Chemical Modifications and Their Biomedical Applications

When referring to a pH-sensitive drug delivery system, PEG is often used in micellar assemblies constituted of a core-shell structure (Fig. 1.5). The main advantage of using PEG in such systems is the fact that PEG is highly hydrophilic, enabling enhanced permeability and retention (commonly referred to as EPR effect) in the bloodstream (Kale and Torchilin 2007; Lang et al. 2019). The EPR effect is responsible for making the core-shell structures to flow in the bloodstream for longer times, which increase the possibilities to the delivery the drug into the specific target. Also, PEG is not recognized as a foreign body by macrophages of the immune system, which enables it to keep in the bloodstream for extra time compared to other polymers (Zambanini et al. 2017). By being held in the bloodstream for a longer time, the drug delivery system can reach the target organ and be even absorbed by cells of a specific tissue. Besides, such stability in the bloodstream enables the usage of PEG-based drug delivery as an injectable system.

As aforementioned, PEG is not a pH-sensitive polymer by itself, which means that its chemical structure lacks in ionizable functional groups. However, three different strategies can be used to transform PEG-based systems into pH-sensitive ones:

(a) Produce copolymers containing anionic or cationic polymer chains bonded to PEG chains.
(b) Add chemical modifications into the PEG structure.

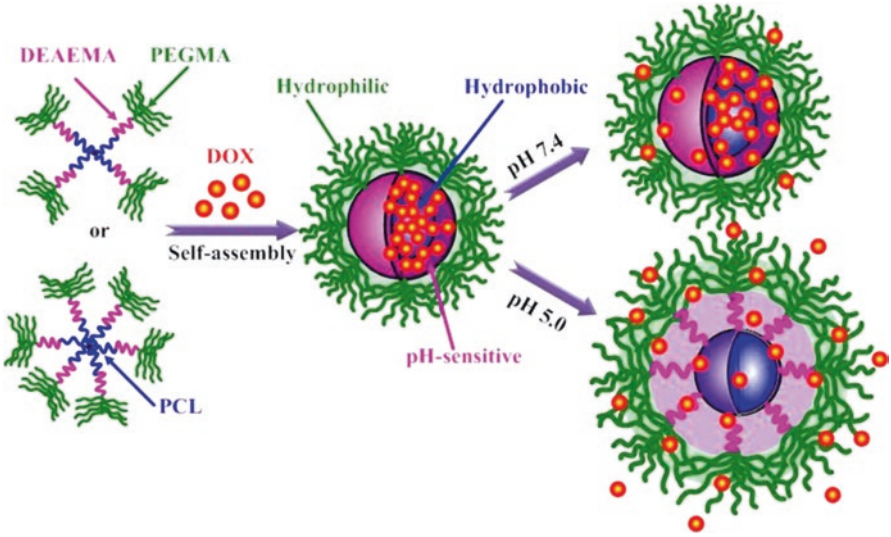

Fig. 1.5 Example of a core-shell structure that contains PEG in a triblock-polymer structure with PCL (poly(ε-caprolactone)) and PDEAEMA (poly (2-(diethylamino)ethyl methacrylate)). This later portion is pH-sensitive. The amino groups in the PDEAEMA structure, the core-shell complex, become pH-sensitive at low pH, when the PDEAEMA structure is destabilized, and the drug is released. Note that in the core-shell complex, the PEG portion is kept in the outer part, enabling the system to take advantage of all of the PEG biological properties. (Yang et al. 2013)

Regarding pH-sensitive PEG-based copolymers, peptides are often used as the blockchain containing the ionizable chemical. Peptides are made of amino acids, while amino acids are organic molecules containing an amine ($-NH_2$) and carboxyl groups (-COOH) in their structure. Then, because of the deprotonation of the amine and the carboxyl groups at different pH, peptides may display a more cationic or anionic polymer behavior depending on their structure. For example, arginine, histidine, and lysine are positively charged amino acids, and consequently, their polymers counterparts display a cationic polymer role in copolymer structures. On the other hand, aspartic acid and glutamic acid are negatively charged amino acids, and their polymers counterparts display an anionic polymer role in copolymers.

There are several works which employed PEG-based copolymers employed as block, as grafted, or even as a combination of block and graft. For example, in work carried out by Lim et al. (2019), both strategies – graft and block copolymer – were used to produce a carrier system to deliver DOX (doxorubicin) and chlorin e6 into cancer sites. To do so, they produced a drug delivery system based on an ionomer polymer that was a result of complexation of two copolymers. The first was composed of PEG-PLL(-g-Ce6) [chlorin e6 grafted poly(ethylene glycol)-poly($_L$-lysine)], and PEG-PLL composed another system (-g-DMA)-PLA [2,3-dimethylmaleic anhydride grafted poly(ethylene glycol)-poly(L-lysine)-poly(lactic acid)]. Note that both copolymers are made of a diblock or triblock main chain grafted with another block. Because of the ionizable chemical bonds found in

the PLL (poly-l-lysine) – that is, a cationic polymer – the carrier system was able to collapse at pH around 6, where the chemical bond between the dimethyl maleic anhydride and the PLL is broken, and the drug is released.

While the block bonded to PEG is responsible for triggering the delivery of a drug, it is not only the unique property related to the drug release kinetics. Note that the pH sensitivity is an ability only related to the chemical structure of the host block, but it will not govern the release kinetics. The drug delivery kinetics will depend on the length of the host block and its chemical composition. Such effect was very clear in a work carried out by Mostoufi et al. (2019), who studied the release kinetics of paclitaxel from series of hybrid diblock copolymers methoxy-poly(ethylene glycol)-b-poly(γ-benzyl-L-glutamic acid) (PEG-PBLG) and triblock copolymers of poly(ethylene glycol)-b-poly(L-glutamic acid-co-NULL-leucine) (PEG-PGA-PLeu). In such study, the glutamic acid chains are ionizable at acidic pH; the authors changed the length of the leucine chain, which is not ionizable. It was noted that a higher pH responsiveness was correlated to the longer hydrophobic non-ionizable segment, Pleu (Fig. 1.6).

The chemical modification enables the adding of functional chemical groups or ionizable molecules in a polymer structure. It is a strategy commonly used to turn non-ionizable polymers into pH-sensitive by introducing ionizable species

Regarding functionalization, the structure of PEG can be modified by adding COOH or NH_2 group at the end of the PEG chain (Zhang et al. 2019). The pH-sensitive hybrid system, composed of graphene oxide (GO) and PEG-COOH as a coating agent, showed to be an effective drug delivery system to carrier doxorubicin (Zhang et al. 2019).

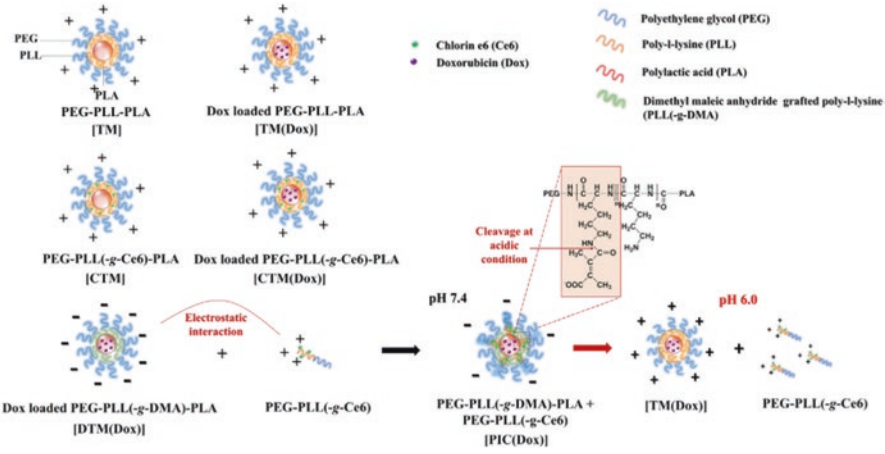

Fig. 1.6 Ionomer polymer composed of two copolymers PEG-PLL(-g-Ce6) and PEG-PLL(-g-DMA)-PLA. The copolymers were prepared in separate and then complexed together to carry doxorubicin and Chlorin e6. When the complex reaches a pH lower than the normal pH of the human body (7.4), the chemical bond between the DMA and PLL is broken, which results in a collapse of the complex structure. Afterward, the drugs are released in the target site. (Lim et al. 2019)

Concerning chemical modification using molecules, usually, ionizable molecules are used to crease acidic or basic liable bonds, which are bonded to a specific drug. Then, when the drug delivery system reaches the target tissue, the drug is released. For example, in work carried out by Chen et al. (2019), the author produced a drug delivery system based on monomethoxypoly(ethylene glycol)-poly(L-lysine)-graft-dimethyl maleic anhydride (PEG-PLL-DMA). The chemical bond between DMA and PLL is liable at slight acid condition, leading to the formation of an NH^{3+} species when the system reaches pH around 6.5 and DMA is also released in the medium.

The applications of pH-sensitive polymers are often focused on the delivery of drugs into tissues that exhibit pH different from that of the physiological fluid (White et al. 2017). In this case, the most used application is on cancer treatment. The cancer cell has low extracellular pH and, consequently, higher intracellular pH compared to healthy cells. Then, many types of research have used pH-sensitive systems to delivery doxorubicin, paclitaxel, and azoreductase, among other drugs (Chen et al. 2019; Cui et al. 2019; Ma et al. 2019; Mostoufi et al. 2019; Yang et al. 2019). In addition, because of the higher specificity of pH-sensitive drug delivery system, they are often used allied to other therapies like photothermal therapy and chemotherapy or used with luminescent molecules that enable the combination of therapy and diagnostic (also known as theranostic) (Liu et al. 2019; Pei et al. 2019; Zhang et al. 2019). For example, in a work carried out by Pei et al. (2019), they produced a drug delivery system based on emulsion copolymerization of glycidyl methacrylate (GMA), poly(ethylene glycol) methyl ether methacrylate (PEGMA), and N-rhodamine 6G-ethyl-acrylamide (Rh6GEAm) with N,N-bis(acryloyl)cystamine) (BACy) as disulfide cross-linker, followed with conjugating DOX via an acid-labile hydrazone linkage (Fig. 1.7). The acid-labile hydrazine linkage enables

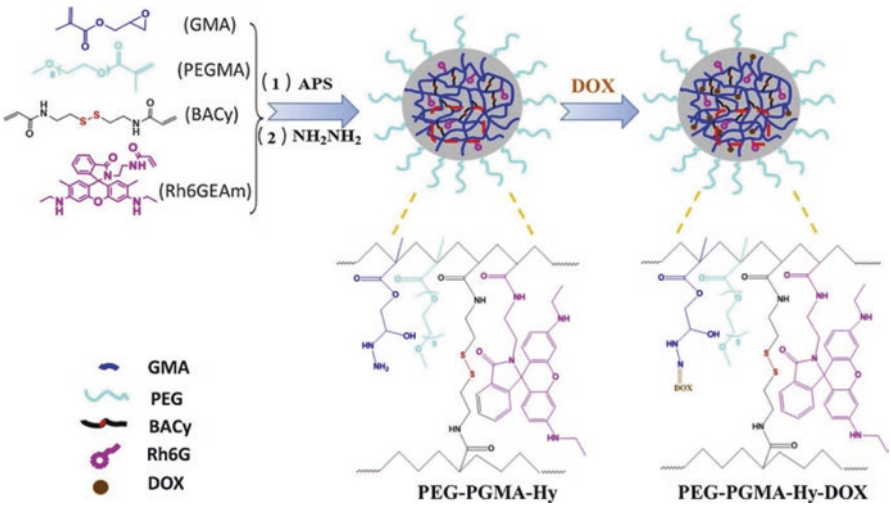

Fig. 1.7 Schematic representation of PEG-PGMA microspheres and PEG-PGMA-Hy-DOX prodrug microspheres. (Pei et al. 2019)

the release of DOX only at acid pH, while Rh6G emits fluorescence only at acidic pH. Then, such drug delivery could be used as a theranostic due to its ability to treat cancer and produce detectable fluorescence.

Another exciting application of pH-sensitive drug delivery systems includes the utilization of a system with bactericidal properties. Bacteria biofilms usually exhibit pH different that of the physiological body fluid, which can be used as a strategy to target them. For example, Zhao et al. (2019) studied a carrier system to delivery chlorhexidine (CHX) in cariogenic biofilm. The drug delivery system was based on cationic poly(ethylene glycol)-block-poly(2-(((2-aminoethyl)carbamoyl)oxy)ethyl methacrylate) (PEG-b-PAECOEMA), and PAECOEMA was modified by citraconic anhydride (CA), forming negatively charged PEG-b-PAECOEMA/CA. The citraconic amides of PEG-b-PAECOEMA/CA block copolymer cleave in acidic medium and accomplish negative to positive charge conversion in a short time. The drug delivery system showed to be effective against *Streptococcus mutans*, and the cytotoxicity of CHX was reduced because of the micellar structure.

3 Conclusion and Prospects

PEG-based materials are one of the most investigated matrices in the fields of thermo- and pH-sensitive systems. Important advances have been achieved particularly on the development of drug delivery systems and tissue regeneration. However, the systems components choice and their synthesis control are the driving conditions for obtaining appropriate structural organization and mechanical properties considering the biomedical applications proposed. The most used techniques for characterizing those systems are small-angle scattering (X-ray and/or neutrons) and rheology. By controlling the hydrogels phase organization and viscoelastic properties, it is possible to obtain systems capable to modulate the drug release rate, dissolution kinetics, bioadhesion, and the in vivo sol-gel transition process. The conjugation of PEG with different synthetic polymers described in this chapter allowed the production of hydrogels systems responsive to physiological (as injectable temperature-sensitive hydrogels) or physiopathological conditions (e.g., pH-responsive hydrogels proposed as therapeutic strategies for acid biological environment). Other exciting potential for the use of PEG-based hydrogels is its approval by FDA, highlighting the potential safety of PEG-derivatives copolymers in biomedical applications. Additionally, all matrices developed must be controlled regrading the synthesis process, physicochemical characterization, components compatibility, and local or systemic toxicity, being of high interest in the fields of biotechnology, biomedicine, engineering, and medicine.

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Chapter 2
Viral and Nonviral Drug Delivery Systems for Medical Health Care: An Overview

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Contents

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1 Introduction

At the beginning of the twentieth century, when the world of gene therapy and therapeutic drugs was at its peak, the quest began to find suitable carriers for their delivery in in vivo systems since the solitary administration of functional genes or the drug proved highly disadvantageous. Rapid clearance from the system, degradation by enzymes, poor solubility, low bioavailability, and inability to reach the target destination for action, all accelerated to the worldwide research for the development of carriers for these compounds of therapeutic importance. However, choosing a suitable delivery system seemed far from easy since there were a set of conditions to be abided by before any entity can be labeled as a medium for delivery. These included size, stability, their drug-loading capacity, and the proper release of the drug at the target site among other basic criteria for choosing a vector for a drug or a genetic material. Depending upon the nature of the molecule serving as the medium for delivery, delivery vectors are conveniently subdivided into two main categories, viral vectors and non-viral vectors. As the name suggests, the former group comprises of the viruses in their nonpathogenic forms, while micro- and nano-sized particles, dendrimers, liposomes, and niosomes all come under the group of non-viral-mediated delivery systems.

Viruses, which are regarded as intermediate between the living and the nonliving, owing to their ability to reproduce only within host cell but not otherwise, make them an excellent choice for formulation of a suitable vector. The process was initiated with the discovery of viral transduction, a mechanism by which virus can efficiently transport their genomes within the cells they infect, in 1952 by the scientists duo Norton Zinder and Joshua Lederberg. These virions are able to target specific hosts and can successfully incorporate and integrate their genome with that of the host's while ensuring stability. Their genomic composition can also be effortlessly amended with intent to insert or delete its functionalities, as needed (Yang et al. 2018).

Alternatively, just as viral entities were being developed as suitable vessels for the transport and subsequent delivery of medicinally important compounds or RNA and DNA strands, its non-viral counterparts were not much behind either. Spherical vesicles, enclosed by single or more lipid bilayers, termed as liposomes, were being designed for similar purposes. However, in spite of simple methods of formulation and organization, liposomes had frequent problems of destabilization, which cannot be disregarded, and the demand for a better and more full-proof medium for the transport of its contents was still present. Soon, after Dr. Richard Feynman introduced the idea of nano-sized particles to the scientific world, the field of drug delivery found a suitable successor to liposomes that overcame the demerits of its predecessor with success. In this chapter, we discuss the various types of delivery systems that are currently being used or in research, their necessities, various diseases against which they are being targeted, and the future prospects they hold.

2 Necessity for Delivery Vectors in Biological Systems

Investigating the essentialities of delivery agents for therapeutic drugs in in vivo settings generated various aims that advocated such extensive research on the field. Some of the pivotal points among them are listed up as below:

2.1 Physical Protection

It is of common knowledge that the entry routes of our body are guarded efficiently anatomic and physiologic barriers. Though these lines of protection originally intent to defend our body for pathogens and harmful substances like toxins or allergens, being nonspecific, the protection is extended to anything that is foreign to the system, including any external drug and/or genes. Thus, sending the therapeutic compounds or genetic materials, in its naked form would almost surely result in its destruction soon after its entry into the body. The delivery vectors, by enclosing the medicinally important compounds within themselves, ensure their protection from the body's immune systems and degrading enzymes.

2.2 Targeted Delivery

Successful administration of any drug of choice must be followed by reaching of the compound to its targeted destination. Failure to do so not only defeats the entire purpose of the therapeutics but also increases the chances of healthy tissues and cells getting affected adversely. In order to prevent that from happening, it is of utmost importance that the drug/genetic material reach the site of pathogenesis. Vessels or carrier systems have shown encouraging results in that front by accompanying the contents to that site, evading distractions on its way. This is generally achieved by the process of active targeting where the carrier system is coupled with a specific ligand with an intent to facilitate interaction with the antigens exhibited on the exterior of diseased cells (Fig. 2.1), or via passive targeting, where the carriers breach through the gaps of the blood vessels at the site of pathogenesis (Fig. 2.2).

2.3 Sustained Release

After addressing the issue to targeted delivery, another aspect of importance that remains is that of sustained release. This is not particularly applicable in case of gene therapy where the delivery vehicles, carrying the gene, insert it all at once into the target cells. However, drugs must be released slowly, for a required interval of

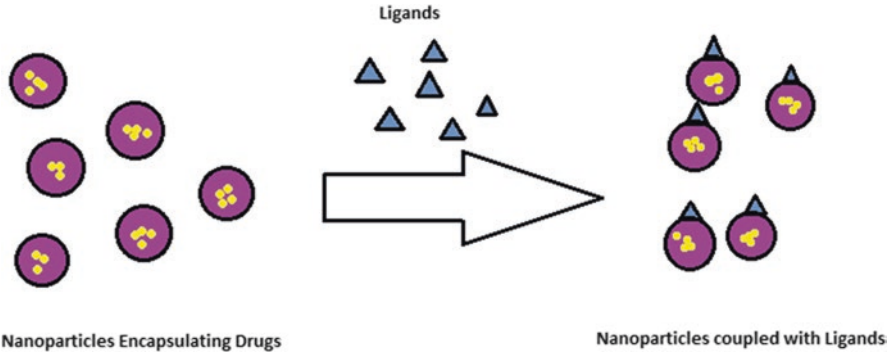

Fig. 2.1 Active targeting by coupling specific ligands with nanoparticles encapsulating therapeutic drugs

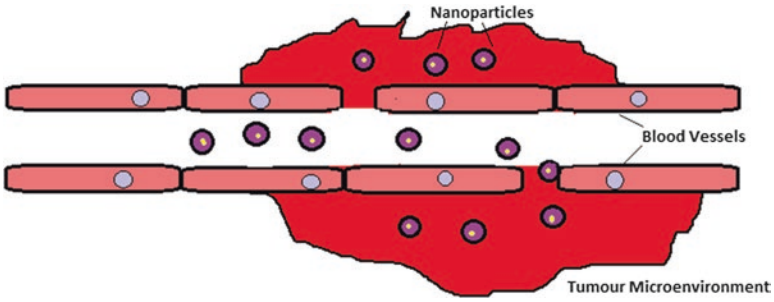

Fig. 2.2 Passive delivery of drugs encapsulated in nanoparticles via enhanced permeability and retention (EPR) effect

time. If the drug is administered without a suitable carrier system, the entire load is exposed at once and is soon removed from the system based on its half-life period. Alternatively, when administered with a delivery agent, the compound is released slowly from the carrier, providing a longer duration for the drug to act and discard the prerequisite for multiple and frequent administration. However, the interaction of the encapsulated or embedded drug with the matrix of the delivery vector should be regulated carefully to make sure that the drug is not so loosely bound to the vectors to allow its premature release nor as tightly bound that the drug fails to leave its carrier and exhibit its therapeutic functions. The kinetics governing the release of the drug should be studied before it is applied in vivo or in vitro. It can be regulated depending upon the type of vector that is being used, its material, the drug of choice, the disease against which it is being targeted, etc. For example, since tumor microenvironments harness acidic pH, nanoparticles carrying antitumor compounds are designed in a manner such that their binding with the drug is weakened in lower pH ranges.

2.4 Regulated Rate of Clearance

Even though it is quintessential for medicines or therapeutic drugs to be available in the bloodstream for a substantial amount of time to exhibit their action, prolonged presence of it might in turn cause damage to the healthy tissues or cells. Thus, as importantly it is to be retained in the body for a required amount of time, it is also necessary that they are eliminated in the due process. Enclosing or coupling them with carrier systems ensures their regulated delivery from the system once their function has been executed to prevent unnecessary drug load in the blood and related toxicity.

3 General Overview of Viral Delivery Vectors

Viruses are microscopic entities that lie on the boundary of the living and the non-living. When outside the host cell, its behavior replicates that of an inanimate object, without response to any external stimuli or any trait of a living being. However, when inside a host cell (can be bacterial, plant or animal), it readily replicates to form numerous progenies, exhibiting one of the most primary features of the living. Almost all viruses have specific hosts, inside whose cells it undergoes reproduction. In nature, virions are considered as potent carriers of gene capable of transferring biological information as a mode of their replication. Wide varieties of viruses are being used as delivery vehicles with variable properties. The most widely employed viruses in drug and gene delivery include adenoviruses, adeno-associated viruses, lentiviruses, retroviruses, and bacteriophages.

3.1 Adenovirus

The adenoviruses are viral entities with icosahedral nucleocapsids housing double-stranded DNA as their primary genetic material. The members of family *Adenoviridae* are characterized by the absence of envelopes, with their size generally ranging from 90 to 100 nm along with having a wide range of vertebrate hosts. In 1953, isolated from the human adenoid cell culture (hence, the name) and until this day, 51 distinct serotypes of it have been acknowledged by virologists all around the globe.

Adenovirus (AV)- and adeno-associated virus (AAV)-mediated delivery systems had been a part of many successful gene therapy procedures, which require the viral-mediated vectors to reach all target cells all over the body. To achieve this systemic delivery of the genes, the initial step should be the infusion of viral vector into the bloodstream. An early study on adenoviral deliveries conducted in 1992 has

claimed successful transfer of a certain gene to the striated muscles of neonatal murine model. This was accomplished by giving intravenous injections to 2–5-day-old mice. However, though the vector was efficacious in reaching various organs and tissues, the rate of transduction in the heart was approximately around 0.2% in contrast to the standard target of gene transfer efficiency, which was 20–50%.

During the last couple of decades, AAVs have emerged as the most prominently used viral vector for gene-based therapies in both human and animal models (Samulski and Muzyczka 2014; Muzyczka and Berns 2015). Additionally, it has been proved its robustness over AVs even after a year of administration without any major immunological response. The remarkable success in rodents had encouraged scientists to scale up the process to use this method of treatment for human models, as well (Duan 2016).

3.2 Retrovirus

One of the widely studied and documented classes of viral particles, one of whose member is known to cause the deadliest infection of acquired immunodeficiency syndrome or AIDS, is the retroviral entities. Despite its ill repute for causing pathogenicity, the retroviral family has also shown considerable potentials as vectors for delivery of molecules of therapeutic importance. The enveloped virions have their sizes around 100 nm in diameter and contain two identical single-stranded RNA molecules of 7–10 kilobases in length as their primary genetic material, which can be converted to corresponding DNA by the action of the enzyme reverse transcriptase. The strand of deoxyribonucleic acid then integrates itself with the genome of the host cell DNA, taking the cell hostage and using it as a site of its own replication.

Retroviruses, just like the other members of its viral family, are primarily used for gene therapy with an intention to transfer and recombine external genetic material into the cells of the lungs, kidneys, heart, liver, or other organs to cure diseases and infections. The major reasons why retrovirals are considered as efficient vectors of gene therapy include its ability to transfer and integrate its genetic material with the host cell genome with stability. Using retrovirals also give researchers the ability to influence target specificity just by altering the heterologous envelope proteins. Three classes of retroviruses that are most popularly opted as vectors for gene therapy include lentiviruses (derived from human immunodeficiency virus or HIV), gammaretroviruses (derived from murine leukemia virus or MLV), and spumaviruses (derived from human foamy virus or HFV). Alongside, there are also alpharetroviral vectors exhibiting a relatively neutral integration pattern that can be used for the purpose of genetic therapies (Suerth et al. 2010).

3.3 Designing a Retroviral Vector

The principle underlying the designing of a viral vector includes recognition of the viral elements or genes that are crucial for the transgene delivery and deletion of the remaining sequence to make room for the gene of interest (GOI). The following step involves providing the essential genes for vector production on separate plasmids to yield a round of infectious viral particles. A detailed, in-depth understanding of viral genomics is essential for the entire procedure. Members of retrovirus genera share an overall similar genetic structure that includes gag, env, and pol genes. While the env code for the envelope of the virion, both gag and pol have structural roles in the viral life cycles.

3.4 Phage Virus

The bacteriophage or the phage, as it is commonly called, is a class of viruses that varies significantly in composition of their envelopes, shape, and size, along with the genetic fragments they house. The term "bacteriophage" indicates toward their exclusive choice of hosts, which are bacteria and archaea only. This natural animosity of these virions against bacteria makes them an excellent choice for numerous therapeutic procedures, collectively termed as "phage therapy." This characteristic of phage particles also makes it the safest option to be employed as medium for delivery since they are incapable of stimulating any immune responses or causing any infection or cancer by itself to mammalian or human cells. They are also capable of condensing the DNA in them to give a compact packaging.

Bacteriophages can be formulated as nano-sized carriers for the targeted delivery of both diagnostic reporter molecules and therapeutic agents. The ligand attached to the phages ensures site-specific delivery of the nucleic acid fragment (DNA, miRNA, etc.)/drug they are carrying, and they can be effortlessly manipulated genetically to carry large quantities of the drug or longer DNAs. Phage-based nanocarriers have been researched extensively against both microbial infections and malignancy (Karimi et al. 2016).

3.5 Filamentous Phage

Filamentous phages are characterized by a distinct structure where a long rod-shaped protein coat encloses single-stranded DNA (ssDNA). These phages are capable of infecting their host cells without killing them by integrating their DNA into the host's genome. Filamentous phages mostly infect gram-negative bacteria like species of *Pseudomonas* and *Escherichia*. The size of these phages depends upon the length of the ssDNA present at its core and can be modified easily by the

deletion and insertion of bases. The mode of entry of this phage-based nanocarrier into eukaryotic cells is endocytosis. Since they are highly immunogenic, the property can prove to be quite advantageous to be used in vaccination. Research in this field has been successful in producing vaccines against HIV-1 and malaria parasites. They have also generated therapeutic antibodies against malignancy and Alzheimer's disease. Filamentous phages displaying HIV epitopes, which are derived from the hepatitis B virus, are adapted to protect organisms against both the diseases. Another application of this class of phages is being investigated for the transport and delivery of antibiotics (Tanaka et al. 2011; Vaks and Benhar 2011).

3.6 Lambda Phage

Discovered by Esther Lederberg in 1950, lambda phage belongs to the coliphage family and is capable of undergoing both lysogenic and lytic cycles. It consists of three parts: the capsid (head), the tail, and a double-stranded DNA sequence as the main genetic material enclosed within the capsid. Lambda phage is generally used in gene therapy, as a nanocarrier for delivery of genes. A recent study introduced the capsid of the lambda phage as a predesigned nanoparticle, and it was incorporated with several different varieties of synthetic moieties and genetically integrated peptides that were exhibited on the phage surface simultaneously (Chang et al. 2014). Another study demonstrates that the encapsulation of the genes encoding for GFP and the E7 proteins of human papillomavirus (HPV) into the lambda vector are capable of enhancing the antitumor immune response countering the prognosis of HPV-expressing cancers (Ghaemi et al. 2010).

4 General Overview of Non-viral Delivery Vectors

Evidently, an advantage that non-viral delivery vectors have over its viral counterparts is the risks from reversion of the nonpathogenic virions to its pathogenic variants. Nonetheless, delivery vessels that are not derivatives of viral sources might need a convenient design, a standardized and regulated method of synthesis among other prerequisites for its smooth functioning.

4.1 Liposomes

Liposomes can be defined as vesicles, which are bound by at least a single lipid bilayer and are spherical in shape. The concept of liposomes (Greek "lipo" meaning fat and "some" meaning body) was first described by the British scientist Alec

D. Bangham in 1961 when he and his colleague R. W. Horne were observing dry phospholipids by negative staining under an electron microscope (Bangham and Horne 1964). In the following year, Bangham, Standish, and Weissmann studied the integrity of the closed, bilayered structure and its property of getting disintegrated and releasing its contents when treated with detergents formed the basis of liposome-based delivery systems (Bangham et al. 1965).

In 1995, liposomes were the first nanoscale medium for drug delivery that was approved for clinical use. Since then research on drug transport systems using liposomes have come a long way aided by great advancements in technology. The lipid molecules constituting the bilayer of liposomes are mostly phospholipids in composition and possess chances of minimal toxicity since these phospholipids are all natural or their derivatives. They are also the most studied nanocarrier systems for targeted delivery of drug. One of the major advantages of employing them is that they are capable of housing both hydrophilic and lipophilic compound without being destabilized. While the polar or hydrophilic molecules are stored within the aqueous center of the lipid vesicles, the nonpolar or the lipophilic ones embed themselves within the lipid bilayer (Hua and Wu 2013). Moreover, these lipid-based structures specialize in carrying not only varieties of drugs but also macromolecules like DNA and polypeptides. Properties like particle size, the charge, composition of the lipid bilayer, number of lamellae, and its surface modification with polymers and ligands can characterize liposomal formulations; all these factors decide their behaviors and stability under in vivo and in vitro conditions (Monteiro et al. 2014).

There are four major types of liposome-based delivery systems, the conventional liposomes, ligand-targeted liposomes, sterically stabilized liposomes, and the combination of the three. In spite of its many advantages, the conventional liposomes were seen to be eliminated rapidly after administration by opsonization from the bloodstream, thus affecting its therapeutic efficacies. To overcome these shortcomings, sterically stabilized liposomes were introduced in which a steric barrier improved the drug efficacy, in both rodent and human models, by preventing opsonization by the serum components. However, soon researchers faced a new problem with this latest design when they realized that the steric molecules that prevented opsonization were also responsible for preventing the essential interaction of liposomes with their target cells. This paved the way for designing of ligand-targeted liposomes to ensure site-specific delivery of drug to particular types of cells or organs in in vivo systems, which selectively express or overexpress those specific ligands or receptors (Hua 2013). Varieties of molecules can function as ligands, like antibodies, polypeptides/proteins, and carbohydrates, depending upon the disease that is being treated, the drug that is being carried, and the phospholipids forming the liposomes. In spite of overcoming the disadvantages of its predecessors, ligand-targeted liposomes have their own limitations like poor immunogenicity and pharmacokinetics. Hence, in the recent years, all three of these methods have been combined to develop liposomes having all these properties and making up for each one's shortcomings (Sercombe et al. 2015).

4.1.1 Ongoing Research and Clinically Approved Liposomal-Based Delivery Systems in Therapeutics

Liposomes as vessels for carrying medicinally important compounds and molecules have offered immense prospects against numerous ailments in the last two decades. With advancements in research and technology, while many lipid carrier-based delivery systems have already been approved for commercial use, some novel carrier systems have been formulated against pathological conditions and are being subjected to extensive in vitro and in vivo studies. For example, in murine models, studies involving folate, coupled with liposomes, showed enhanced distribution of the latter in folate-expressing neoplasms. In a separate study, immunoliposomal formulation with doxorubicin, functionalized with antibodies targeting the epithelial growth factor receptor (EGFR), has been designed for effectively combating the spread of solid tumors (Mamot et al. 2012). Some of the other liposome formulations that are currently under study are loaded with different types of therapeutic agents, including L-BLP25 peptide for lung carcinoma, irinotecan (MM-398) for pancreatic cancer, and cisplatin (Lipoplatin) for ovarian cancers (Wu et al. 2011; Ko et al. 2013; Casagrande et al. 2014). Apart from the therapeutic liposomes, there are also extensive research being carried on the theranostic and diagnostic properties of them (Xing et al. 2016). Another variety of PEGylated liposome encapsulating irinotecan that is presently in its Phase I trials is PEPO2, which is being used for the treatment of advanced refractory solid tumors (Chang et al. 2015). Besides these, there are also some cation-based lipid carrier systems that have received recent approval from FDA for medical trials.

4.2 Microparticles

With diameters ranging from 1 to 1000 μm, microparticles have shown their efficacy for the delivery of various therapeutic materials that include various medicinal compounds as well as RNA and DNA molecules. Microparticles, having a large surface area-to-volume ratio, show characters which are absent in its bulk form that can be successfully exploited to design a suitable delivery agents, depending upon the item for transport.

5 Microparticles for Delivery of Therapeutic Drugs

Pioneering research on microparticles confirms its impending potentials in pharmaceuticals and biomedics industry. Microparticles can be synthesized from a variety of polymers with an intention of site-specific delivery of various drugs of therapeutic importance. Some of these studies have been discoursed in brief in this section.

Inflammatory bowel diseases (IBD) that are chronic autoimmune ailments affect the gastrointestinal (GI) tract that also leads to ulcerative colitis, Crohn's disease, and colorectal cancer. Recurrent symptoms like abdominal pains, vomits, and bowel distension along with mucus diarrhea are symptoms of IBD due to which frequent administration of anti-inflammatory drugs becomes essential. However, despite that, the efficacies of the anti-IBD drugs are compromised due to their rapid absorption in small intestine region. Moreover, repetitive administration of such elevated doses of anti-inflammatory drugs might also generate some side effects from prolonged use. For such colon-related disorders, alginate microparticles have taken shown great potentials as a medium for delivery of therapeutic drugs, owing to its pH sensitivity. Alginate (salt form of alginic acid or align) is a polysaccharide, which is extracted from the cell walls of brown algae that is hydrophilic in nature, forming a viscous gum when hydrated. Numerous techniques like ionotropic gelation, emulsion, and complex coacervation can be employed to prepare microparticles of alginate. Their characterization, in vivo and in vitro studies have yielded encouraging results indicating toward a promising future for these microparticles (Agüero et al. 2017).

Another recent study investigates the potential of inhalable microparticles against lung carcinoma. This noninvasive method of treatment also enables the organ-specific delivery of chemotherapeutic drugs. Recent advancements in inhalable microparticle-based drug delivery structures for lung cancer therapy include bioresponsive, large, porous, solid lipid, and drug-complex microparticles. Synthetic hydrophobic polymers were initially used for the synthesis of MPs with intent of sustained drug release. However, their low drug-loading capacities and accumulation of synthetic polymer in the lungs after multiple administrations compelled researchers to look for alternatives, which was fulfilled by the natural polymer sodium alginate and chitosan. Paclitaxel (PTX), cisplatin (CIS), and 5-fluorouracil (5-FU) are some of the most frequently used drugs to be coupled with alginate microparticles intended for lung cancer therapy. Dry powder inhalers (DPIs) are a solid-based delivery system that can be engaged for the application of drug-containing MPs to the lungs. DPIs are also advantageous for handling purposes and have lasting stability in storage. However, despite its many advantages, the same study also advocates that microparticles having size range of 3–5 μm have much low rates of internalization by lung cancer cells than nanoparticles with diameter 100–150 nm (Abdelaziz et al. 2018).

6 Microparticles for the Delivery of Genetic Material

The applications of microparticles as vehicles for delivery are not limited to therapeutic drugs alone. Many DNA vaccines, in recent times, have also coupled with MPs made of polymers for their targeted delivery as well as sustained release. These vaccines are extensively used in veterinary sciences but have not yet been approved for use on humans. However, owing to some innovative research and efforts from

some brilliant minds, the day might not be that far when MPs see uses as gene vectors for humans as well. Some of the latest and the most pioneering of these research works have been discussed in brief, in this section.

DNA vaccination has been successfully demonstrated to be effective in several animal models for prevention and treatment of numerous infectious diseases, hypersensitive reactions, cancer, as well as autoimmune disorders. This has propelled the study and research of DNA vaccines in human models as well (Suschak et al. 2017). Microparticles with their size ranging from 1 to 10 μm have been suitable carriers of DNA vaccines to the antigen-presenting immune cells due to preferential uptake and processing of the particles of that size by the APCs. The polymeric microparticles carrying the DNA fragments also provide protection from being degraded by nucleases. The most widely used polymer for microparticles, encapsulating DNA fragments, is poly(lactide-co-glycolide) or PLGA. It has proved its efficacy against malignancy, swine flu, and hepatitis B among other disorders. Additionally, PLGA microparticles enclosing plasmid DNA (pDNA) encoding an antigenic peptide of the human papillomavirus (HPV) are in its Phase II clinical trials and have shown to increase T-lymphocyte responses to HPV epitopes (Matijevic et al. 2011). Another formulation of PLGA microparticles was developed where the surface of the MPs was coated with pDNA (encoding the 1D gene of the foot-and-mouth disease virus) instead of encapsulating it. It showed higher production of FMDV-specific antibody and neutralizing antibody titers, along with increased lymphocyte proliferation in murine models compared to the administration of naked plasmids (Reddy et al. 2012; Farris et al. 2016).

6.1 Nanoparticles

From the aspect of our discussion on delivery vessels, nano-sized particles hold an immense importance in the concerned field. Even though this is a comparatively novel area of study, there are scientists who claim that use of miniature molecules as drug carriers dated back to the Vedic ages in India where the leftover ashes from pyres of religious ceremonies had potential healing powers (Chaudhury 2011).

Speculations aside, there is no denying of the fact that nanoparticles have proved their potential, time and again as robust and competent medium of delivery for carrying drugs to their desired locations and to release them effectively. Nanoparticles, just like microparticles, are possessors of characteristics that are unknown in its bulk forms. NPs can be prepared from materials like natural and synthetic polymers, metals, etc. among others. Nanoparticles yielded from polymers are easily biodegradable and hence have lower chances of causing toxicity even after prolonged and frequent use. Due to their reliable stability in in vivo and in vitro settings and minimal size ranging from 10 to 200 nm, nanoparticles are also capable of blood-brain barriers (BBB), gain access to the pulmonary system, and are also easily absorbed through tight junctions of endothelial cells of skin (Betzer et al. 2017). This enables therapeutic drug loaded in it to reach obscure locations of the body, which was almost impossible earlier with liposomes and microparticles. Another advantage of nanoparticles by virtue of its small size and large surface area is that they are readily

soluble in body fluids, hence increasing the bioavailability of the compounds they sheathe. Medicinal drugs that were initially discontinued for having poor solubility and, thus, low bioavailability were again introduced into biomedicine research to be coupled with nano-sized particulates (Rizvi and Saleh 2018).

7 Nanoparticulate-Based Drug Delivery in Cancer

Cancer or malignancy that is typically symptomized by the formation of neoplasm is one of the leading causes of mortality around the world, second only to cardiovascular disorders. Radiotherapy, chemotherapy, and surgery are some of the most prevailing methods of treatment and to prevent its spread, but in many cases, these conventional therapeutic approaches fail or might manifest serious side effects (Baudino 2015). Nanoparticulate-based approaches to treat malignancy have yielded encouraging results in recent years some of which have even been permitted by FDA for clinical trials and for commercialization. For instance, curcumin, a major component of *Curcuma longa*, has been speculated to have antitumor properties. However, the drug could not be used to its full potential because of its low solubility in bodily fluids. Recent studies using curcumin in hydrogel-based nanoparticles could confirm successful inhibition of necrosis factor-κB and interlukin-6, which have pro-inflammatory roles in pancreatic cancer (Tajbakhsh et al. 2017).

Single- and double-walled carbon nanotubes (SWCNTs and DWCNTs) are another approach to nanoparticulate-based drug therapy where an allotropic form of carbon with cylindrical framework is used. Doxorubicin (DOX), a popular anticancer drug, has been conjugated with CNTs under in vivo and in vitro conditions, which confirms that DOX with CNT demonstrates a more targeted delivery, and is more effective in destruction of neoplastic cells compared to higher doses of free DOX. Furthermore, the sustained release of DOX was seen to be enhanced at lower pH of tumor microenvironments. The needlelike shape of CNTs also elicits transmembrane penetration of the drug by enabling the nanocarriers to transport it to obscure locations in the body that were formerly inaccessible by the drug alone (Dinesh et al. 2016; Sanginario et al. 2017).

8 Nanoparticle-Based Drug Delivery for the Treatment for Tuberculosis

Caused by the bacteria *Mycobacterium tuberculosis*, this airborne ailment kills more than a million worldwide, affecting another billion each year. Initially, rifampicin (RIF) and isoniazid (INH) were the drugs of choice against TB and were recommended as first-line anti-TB medications. However, prolonged and unregulated misuse of these drugs has made the causative organism resistant to the medications, giving rise to multidrug-resistant (MDR) strains of the bacteria. This has compelled researchers all across the globe to search for a novel treatment of choice, and nanoparticulate-based drug therapy has been a significant tool in this war against drug resistivity.

Ethionamide (ETH) is a popular second-line drug against TB, whose applications were initially compromised by its low solubility and shorter half-life. This problem was overcome by using nanoparticles made of polymeric β-cyclodextrins (pCD), biodegradable poly(D,L-lactic-co-glycolic acid) or PLGA, and polylactic acid. ETH-booster pair was co-encapsulated in these nanocarriers increasing not only its solubility but also minimizing the tendency of the therapeutic compound to form crystals in aqueous environment (Costa-Gouveia et al. 2017). Other diseases that are currently using nanoparticulate-based therapeutics include various viral, bacterial, and fungal infections, neurological disorders, psychiatric conditions, AIDS (acquired immunodeficiency syndrome), etc.

9 Hazards Associated with Various Delivery Systems

Whenever a new discovery or invention is made, each one comes with its own set of pros and cons. In viral delivery vectors, it is of utmost importance that pathogenic genes are deleted before administering it into the body. Even then, reversion of those viruses to its pathogenic strains is not a very uncommon phenomenon. The problem is further complicated if the vector is being commercialized. Under such large-scale productions, even the reversion of a single virion can cause huge damage. Non-viral delivery methods, even though they do not pose such threats, have a few drawbacks of its own. Repeated administration of nano- and micro-sized particles may cause hazards, if they are not cleared from the system at regular intervals or metabolized. If the substance from which the particles are being synthesized is inorganic in nature, like metals or synthetic polymers, then the risks of accumulation in the system are also elevated. Even by using natural polymers like alginate or chitosan, the chances of hypersensitivity or individual reaction to those compounds cannot be eliminated. However, comparing the pros and cons of both types of delivery systems, non-viral methods easily gain triumph over its viral counterparts; easy elaboration, minimal cytotoxicity, lack of oncogenic effects, ability to house larger DNA fragments, and immune tolerance, all advocate to the preference for non-viral carriers than virions acting as vectors in biological systems.

10 Latest Trends in the Field of Vector-Mediated Delivery Systems

In the preceding sections, we have discussed some of the conventional approaches of drug and gene delivery vectors that are being used extensively. However, in the recent years, there have been some new formulations with which the delivery of genes and drugs have taken a leap forward and hold a promising future in the field of therapeutics and theranostics. Discussing some of these recent advances, here are some novel modes of delivery that are presently being studied and researched.

10.1 Niosomes

Primarily used for gene delivery, niosomes are liposome-like, bilayered structures that are composed of lipid molecules. The major factor that differentiates it from conventional liposomes is that the lipid moieties constructing it are all non-phospholipids (Attica et al. 2017). The lipid molecules that are used for the formulation of niosomes are cationic or positively charged and form complexes (nioplexes) with the genetic material bearing negative charge (Agirre et al. 2015). Some major advantages that they offer over liposomes are that the method of synthesis is much cheaper and also that they can be stored for a longer duration (Rajera et al. 2011) along with better penetrating capabilities. Apart from the positively charged lipid molecules, nonionic surfactants are used to ensure stability and refrain the particles from aggregating, along with helper lipids that enhance the physicochemical features of the emulsion, hence improving the efficiency of gene delivery. Numerous methods can be employed for the preparation of niosomes, depending upon convenience, cost, and the components that are being used; reverse-phase evaporation (REV) methods, microfluidization method, and proniosome technology (PT) are some of the prominent of those.

A recent study demonstrates the ability of the niosomes of crossing the blood-brain barrier that can be used for the development of novel carriers for gene therapies for diseases of the central nervous system. Niosomes synthesized with DOTMA cationic lipid, polysorbate 60 as nonionic surfactant, and lycopene as helper lipids were tested under in vivo conditions of rodent brain, which showed good transfection efficiencies followed by significant protein expression in the cortical glial cells, without compromising on the viability (Mashal et al. 2018).

A separate research evaluates the effects of chloroquine diphosphate on a niosome formulation of poloxamer 188, polysorbate 80 as a nonionic surfactant, and 2,3-di (tetradecyloxy) propan-1-amine (hydrochloride salt) cationic lipid on rat retina for the treatment of retinal degeneration. Two varieties of niosomes are prepared, one of them with and another without chloroquine by the process of reverse-phase evaporation technique, and are incorporated with plasmid pCMS-EGFP to form the respective nioplexes. In both in vivo and in vitro conditions, the transfection efficiency and subsequent protein expression were improved in the former (Mashal et al. 2019).

11 Conclusion

We hence come to close our discussion on the numerous types of delivery systems that are presently in use in the field of therapeutics and biomedicines as vectors for targeted delivery and sustained release of drugs and genetic fragments. Apart from the ones discussed, there are many other novel methods, which are currently being studied; some of them have been mentioned and discussed in Table 2.1 Alongside, there are vectors that have already received approval for clinical trials and for com-

Table 2.1 Viral and Non-viral Delivery Vectors and their Advantages

Viral delivery vectors				
Type of virus	**Details of the carrying molecule/complex**	**Used against/for**	**Advantages**	**References**
Herpes simplex virus (HSV)	Zinc-finger proteins (ZFPs)	Targeted epigenetic remodeling	Versatile and easily reproducible	Hamilton et al. (2018)
AAV (serotype 9)	Atrial natriuretic factor (ANF) promoter	Cardiac disorders/atrial diseases	Efficient and dose-dependent	Ni et al. (2019)
AV type 5 and AAV type 2	CRISPR/Cas9 gene	Hemophilia B	Cheaper and log-term effectivity	Gao et al. (2019)
AAV	Large-sized genes	Genetic diseases with mutations of large genes	Increased packaging capacities	Patel et al. (2019)
AAV (serotypes 5, 7, 8, 9)	Large-sized genes	Inherited retinal diseases	Increased packaging capacities	Trapani (2019)
Recombinant AAV (serotype 9)	Human ABCD1 gene	Adrenomyeloneuropathy, adrenoleukodystrophy	Efficient gene transfer across the spinal cord, transgene expression and safe	Gong et al. (2019)
AAV	Ligand-binding soluble form of VEGFR3	Meningeal lymphatic vasculature	Low immunogenicity, long-term in vivo expression	Karaman et al. (2018)
AAV	Channelrhod-opsin-2 gene	Investigating the role of neural circuits in behavior	Better gene expression	Yazdan-Shahmorad et al. (2018)
Non-viral delivery vectors				
Base material of delivery system	**Details of the carrying molecule/complex**	**Used against/for**	**Advantages**	**References**
Chitosan	RGD peptide	Ovarian cancer	Stability, precise targeting	Fu et al. (2018)
Cationic lipids/niosomes	RGD peptide	Breast tumor	Efficiency of gene transfer	
Cationic lysine residues	RGD peptide	Pancreatic cancer	Improved specificity and transfection efficiency	

Cationic carbon dots	Plasmid SOX9	Chondrogenic differentiation	High solubility and yield, low cytotoxicity, outstanding biocompatibility and self-tracking ability	Cao et al. (2018)
Sleeping beauty (SB) transposon vector	Minicircle DNA	Correction of hematopoietic stem and progenitor cells (HSPCs)	Better transfection efficiency, cost-effective and safe	Holstein et al. (2018)
Supramolecular nanovesicles of β-cyclodextrin derivative and liposomes	Adamantyl guanidines and plasmid DNA	–	Improved efficiency	Štimac et al. (2019)
Solid lipid nanoparticles (SLNs)	Aripiprazole	Schizophrenia	Enhance in vivo efficacy and oral bioavailability of the drug	Silki and Sinha (2018)
2,3-di(tetradecyloxy)propan-1-amine niosomes	Bone morphogenetic protein-7 gene	Bone regeneration	Increased growth rate of the bones	Attia et al. (2018)
1,2-dioleoyl-3-trimethylammonium-propane niosomes modified with hyaluronic acid	pEGFP-C1 plasmid	Gene delivery to retinal pigment epithelium	Efficient gene transfection, higher cell viability	Qin et al. (2018)
Cationic niosomes	Human bone morphogenetic protein (hBMP7) gene in pUNO1-hBMP7 plasmids	Brain injuries and glioma	Improved migration to glioblastoma cells	Attia et al. (2019)
Chitosan (in native and in modified forms)	Insulin	Diabetes mellitus	Oral intake, protection against acid degradation in the GI tract	Wong et al. (2017)
Alginate, dextran sulfate, chitosan, and albumin (multilayered)	Insulin	Diabetes mellitus	Reduce the basal blood sugar level	
Dextran-vitamin B12	Insulin	Diabetes mellitus	Increased biodegradability and biocompatibility	

mercialization but are being studied relentlessly for improvements. The choice of a suitable vector might depend on several factors, for instance, the content that is being delivered, the ailment against which it is being targeted, whether the delivery is site-specific or systemic, and most decisively the pathological and immunological condition of the individual on which the treatment is applied. This further ignites the issue of personalized medicines and its relevance in today's world. There is no denying that whatever mode of delivery is chosen, the menaces of hypersensitivity or reversion to pathogenicity for viral vectors cannot be eliminated entirely. Only via extensive research can we hope to conquer the shortcomings of each of the methods.

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Chapter 3
Nanotoxicology in Plants

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Contents

1 Introduction

> Paracelsus (1493–1541): "dosis sola facit venenum."
> "The dose alone makes the poison."

The etymological roots of the term toxicology stem from the Greek words τοξικον (toxikon = poison) and λογια (logia = treatise or science). Toxicology, therefore, was defined as the field of science responsible for the study of poisons.

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Current research largely focuses on elucidating and describing the potential effects of different substances and materials considered harmful to living organisms. Other subfields of toxicology are related to nature, incidence, dose-response relationship, cellular uptake, severity (genetic, cellular, systemic, and immunogenic, among others), reversibility, and mechanisms of action of compounds and to response mechanisms in the organism (Burcham 2014; Murphy 1979). Conversely, the prefix nano (νανοζ), which originally meant dwarf, is currently used in the International System of Units to indicate a factor of 10^{-9}.

Thus, nanotoxicology is defined as the branch of toxicology responsible for describing the effects of nanomaterials (or the nanometric scale) on living organisms, considering their physicochemical characteristics, dose-response relationship, cellular uptake, mechanisms of action, severity, and reversibility, among other variables.

2 Nanomaterials

To standardize terms in scientific and industrial contexts, at least two definitions of nanomaterials have been accepted for regulatory purposes. First, the definition adopted by the International Organization for Standardization/Technical Specifications (ISO/TS) 80004-1:2015 (ISO 2015) establishes that nanomaterials are those materials with at least one of their dimensions (internal, external, or surface) on the nanoscale (1–100 nm). In turn, the definition published by the European Commission in 2011 (2011/696/EU) considers a nanomaterial as "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 is in the size range 1 nm-100 nm" (European Union Commission Recommendation 2011).

Nanomaterials have gained considerable relevance in industrial fields as components of paints, cosmetics, rubber, additives, electronics, environmental remediation devices, tools, textiles, and sports equipment. In the biomedical field, nanomaterials are mainly used in drug release, imaging, implant coatings, and aseptic methods (Roszek et al. 2005); in agriculture, they are used for the controlled release of herbicides, pesticides, and fertilizers. Currently, nanomaterial engineering research has gained relevance (Caballero-Guzman and Nowack 2016; Charitidis et al. 2014; Joo and Zhao 2017). In all the aforementioned areas, properties such as electrical conductivity, high resistance, structure, electronic affinity, catalysis, photo-optical, and electronic characteristics, functionalization, biocompatibility, flexibility, and malleability are used for specific purposes, and many nanomaterials are produced on a large scale, which has increased their presence in the environment (Goswami 2017; Peralta-Videa et al. 2011).

One of the disadvantages of nanomaterials is their potential toxicological-environmental effects. It has been shown that, due to their physicochemical characteristics (size, surface area, shape, chemical composition), nanomaterials have stronger toxicological effects than those reported for the same materials in bulk or

in solution (Joo and Zhao 2017; Stone et al. 2010). Most studied nanomaterials have shown dose-dependent toxicity, which is directly related to the availability of the nanomaterial in the environment (culture medium, air, water, soil, and food, among others), to the mechanism of cellular uptake or penetration, and, lastly, to the duration of contact with the nanomaterial (Buzea et al. 2007; Krug and Wick 2011; Navarro et al. 2008). The primary toxicological factor observed in various organisms, regardless of the nanomaterial, is oxidative stress, a process by which the production of reactive oxygen (ROS) and nitrogen species (RNS) is induced. Cellular damage, such as apoptosis, mitochondrial damage, plastid damage, autophagy, and genotoxic effects due to deoxyribonucleic acid (DNA) fragmentation and chromosomal abnormalities have also been reported. In tissues, the damage depends on the type and amount of the captured nanomaterial and the exposed tissue, causing histological changes, cell growth inhibition, carcinogenic and/or teratogenic effects, and even death (Buzea et al. 2007; Gerloff et al. 2017; Lewinski et al. 2008).

2.1 Classification of Nanomaterials

There is no single classification of nanomaterials. Depending on the authors and research areas, aspects such as the chemical basis (organic or inorganic) (Mageswari et al. 2016), origin (natural or artificial), and physicochemical characteristics (morphology, volume, surface area, surface charge, crystallinity) are considered for classification purposes. Finally, they can also be classified based on their dimensionality (0, 1, 2, and 3D) (Khan et al. 2019; Lewinski et al. 2008). This chapter will address a classification focused on their chemical basis, albeit mentioning other characteristics.

2.1.1 Carbon-Based Nanomaterials

Graphene materials, fullerenes, carbon nanotubes, and nanodiamonds, among others, are included in this group. Graphene is described as a two-dimensional (2D) sheet of sp^2-bonded carbon atoms arranged in a flat hexagonal lattice structure, similar to a honeycomb (Allen et al. 2010). This arrangement can be modified on its surface, by oxidation-reduction reactions or by functionalization, which directly affects its electronic structure and therefore its physicochemical characteristics and interaction with other materials, including cells. Studies have shown that graphene toxicity is dose-dependent and that sheet size is a determining factor (Montagner et al. 2016; Sanchez et al. 2012). *Fullerenes*, in turn, consist of between 28 and 1500 carbon atoms arranged in box-like structures. Their structures can have up to 120 different symmetries, thanks to the arrangement of their atoms in pentagonal and hexagonal rings. The most symmetrical fullerene is the C60 fullerene, with a size of 0.7 nm and a spheroid shape similar to that of a soccer ball. As with other nanomaterials, C60 fullerenes have a large surface area and, despite their low reactivity, are

susceptible to functionalization, which makes it possible to improve their chemical or biological activity (Isaacson et al. 2009). Carbon nanotubes (1D) have an elongated shape, similar to fiber. They can be single-walled carbon nanotubes (SWCNTs) or multiwalled carbon nanotubes (MWCNTs). As with other allotropes of carbon, they are susceptible to chemical modification, which determines their physical and chemical characteristics and is related to their fate and toxicity potential (Alshehri et al. 2016; Allegri et al. 2016). Nanodiamonds, conversely, are more stable than other carbon-based nanomaterials. To date, studies have reported that these allotropes have low toxicity (Schrand et al. 2007).

2.1.2 Inorganic-Based Nanomaterials

Inorganic-based nanomaterials include nanoparticles (0D) and thin films (2D). Both materials can have a metallic, ceramic, or semiconductor composition. Metallic nanomaterials contain zerovalent compounds such as iron, silver, and gold. They are mainly synthesized from metal salt precursors (Khan et al. 2019). Ceramic materials are nonmetallic inorganic solids produced by heat treatment; they can be found in amorphous, polycrystalline, dense, porous, or hollow shapes (Yamamoto et al. 2004). Semiconductors, in turn, have intermediate properties, that is, between metals and nonmetals. They usually consist of elements from groups II to VI or III to V (Lewinski et al. 2008; Owen and Brus 2017). Inorganic-based nanomaterials are currently produced by controlling synthetic methods for generating these materials with specific morphology, size, charge, coating, and optoelectrical properties. Biocompatibility or biocidal activity has been described for many nanomaterials. Thus, for example, among the toxic mechanisms against various pathogens (bacteria, fungi, and protozoa, among others) (Rodríguez-Torres et al. 2019; Vazquez-Muñoz et al. 2017) identified in many metal nanoparticles (Ag, Cu, and Zn, among others), the release of metal ions that interact with various cellular components has been reported (Krug and Wick 2011). Another significant deleterious effect reported for metallic, ceramic, and semiconductor nanoparticles is mechanical stress on cells or tissues due to the accumulation and/or morphology of the nanomaterial (Yamamoto et al. 2004). Unfortunately, cytotoxic effects are also observed in nonpathogenic organisms, in animal (including human) cells and tissues, and plants.

2.1.3 Quantum Dots

Quantum dots are semiconductor materials formed from elements of groups II to VI or III to V or from carbon-based materials. They are crystalline nanometric structures with a diameter that is smaller than twice the Bohr radius of its exciton (electron hole-electron pair), thereby producing their quantum confinement. These materials have adjustable band gaps depending on the size of the material and on its

crystalline structure, in addition to showing remarkable luminescent properties. Although quantum dots are usually better known, other 1D and 2D crystalline nanomaterials have similar characteristics (Frecker et al. 2016; Owen and Brus 2017; Valizadeh et al. 2012).

2.1.4 Organic-Based Nanomaterials (Biomaterials)

Organic-based nanomaterials (lipids, carbohydrates, and other biopolymers) have been primarily studied in biomedical applications, particularly in the development of nanomaterials for drug delivery. Lipid nanomaterials can be micelles, liposomes, solid lipids, and nanostructured lipid carriers, which are used in the pharmaceutical, cosmetic, and food industries, mainly for molecule encapsulation and delivery to tissues (Angelova et al. 2017; Barriga et al. 2019; Jobin and Netto 2019; Tapeinos et al. 2017). Polymeric nanoparticles are synthesized from cross-linked biopolymers. Alginate, chitosan, gelatin, hyaluronic acid, polylactic-co-glycolic acid (PLGA), polylactide (PLA), polycaprolactone (PCL), and polyanionic cellulose (PAC) are among the most studied polymeric nanoparticles (Kumari et al. 2010; Nitta and Numata 2013). Dendrimers are polymeric molecules with a regular size and geometry and with well-defined and controlled branching via the appropriate selection of materials for their synthesis, thereby defining properties such as size, porosity, cavity type, hydrophobicity, and hydrophilicity, among others (Kesharwani et al. 2018; Kim et al. 2018). With the development of genetic engineering, protein-based nanoparticles have been recently produced from the synthesis of self-assembling protein subunits, which makes it possible to control characteristics such as surface loading, encapsulation, and ligand release, among others (Heddle et al. 2017; Tarhini et al. 2017). To date, few cytotoxic effects of organic-based nanomaterials have been reported, although nanotoxicology remains a relatively young research field.

2.2 Nanomaterial Production

According to some authors, nanomaterials have been naturally produced through processes such as volcanism, hydrothermal systems (Navarro et al. 2008), soil erosion, and significant energy release events such as lightning or meteorite impact (Isaacson et al. 2009). Interestingly, the evolution of living beings on the planet generated the production of nanomaterials from cellular processes, such as the production of nanoparticles from bacteria (Faivre and Schüler 2008; Li et al. 2016) and fungi (Park et al. 2016). The formation of carbon nanomaterials as a result of the combustion of organic materials in forest fires has also been observed (Buzea et al. 2007). With mass production, nanomaterials have become a potential risk. Different sources of production include fossil fuel and agricultural waste burning, internal

combustion engines, chemical-biological waste generation and disposal, and water treatment processes (Bour et al. 2015; Nowack 2017). Recently, material engineering has become highly relevant in large-scale industrial applications, as has research on the controlled production of nanomaterials with a specific shape and size, and with high purity, which are used in a large number of commonly used devices (Koivisto et al. 2017).

2.3 Transport, Distribution, and the Fate of Nanomaterials in the Environment

In nature, nanometric- and micrometric-scale materials are ubiquitously distributed. The wind is one of the most important mechanisms of natural transport of nanomaterials (Joo and Zhao 2017). Propagation through bodies of water not only by surface runoff and ocean currents but also by aerosol formation (also determined by local climatic factors) is another key dispersion mechanism (Gottschalk et al. 2011; Joo and Zhao 2017). In addition, anthropogenic activities, both industrial and agricultural, are an important transport route. Nanomaterials reach the air, bodies of water, the soil, streambeds, and the seabed naturally (due to atmospheric deposition, rain, and surface runoff, among other processes) or due to poor landfill management, via sewage sludge, or through its application in agricultural soils, among other activities (Wigger et al. 2015).

In the environment, nanomaterials may be exposed to various processes that affect their mobility patterns, bioavailability, fate, and toxicity (direct or indirect). Such processes involve homo-aggregation with nanomaterials of the same composition, hetero-aggregation with nanomaterials of different composition or with other molecules, changes in size, shape, surface charge, chemical stability, age, phototransformation, dissolution, interactions with other ions, and interactions and/or transformation with macromolecules and/or biopolymers contained in organic matter (Dwivedi et al. 2015; Goswami 2017).

3 Plants

To understand the toxic effects that naturally or artificially produced nanomaterials may have on plants, this chapter will provide an overview of the general morpho-anatomical and physiological characteristics of plants that affect the mechanisms of the interactions and the ease of uptake from the environment and discuss the resistance and responses of plant cells to nanomaterials. The plant kingdom is diverse; it includes nonvascular plants (bryophytes), seedless vascular plants (ferns, whisk ferns, horsetails, and lycophytes), and seed vascular plants, which are divided into two broad groups: gymnosperms (cycads, ginkgo, gnetophytes, and conifers) and angiosperms,

which produce flowers and seeds (magnoliids and mono- and dicotyledonous plants) (Kaplan 2001).

Plant cells are delimited by a cell membrane (plasmalemma) that consists of phospholipids and proteins, which are coated with a semirigid cell wall with a similar architecture in all plants, composed of cellulose microfibrils, polysaccharides, lignin (only in vascular plants), structural proteins, and enzymes (peroxidases, pectin esterases, extensins, and expansins, among others). They may also contain phenolic compounds, gums, resins, silica, calcium carbonate, suberin, waxes, cutin, and Ca^{2+}. The thickness can vary from a few hundred nanometers to a few micrometers. The composition of the cell wall and membrane varies depending on the species and even on the tissue (Khalil et al. 2010; Lee et al. 2011) (Fig. 3.1a). Adjacent cells are interconnected through plasmodesmata, that is, intercellular channels in which proteins and membranes subdivide cell walls into microscopic channels of 3–4 nm in diameter and that can reach just over 10 nm. Plasmodesmata are contact areas through which water and nutrients flow from or to the vascular system (Fig. 3.1b) (Knox and Benitez-Alfonso 2014; Sevilem et al. 2015). As in other eukaryotic organisms, the cellular cytoplasm has networks of microtubules and organelles, such as the endoplasmic reticulum, the Golgi complex, mitochondria, the cell

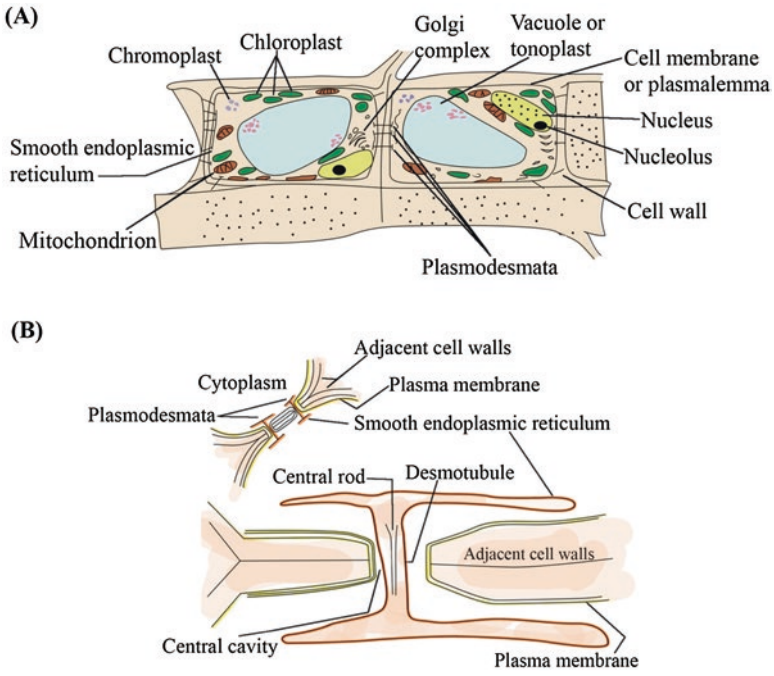

Fig. 3.1 (**a**) Diagram of the main components of a plant cell. (**b**) Diagram showing that plasmodesmata are interconnection zones between adjacent cells. In each cell, a portion of the endoplasmic reticulum, termed the desmotubule, runs through the center of the plasmodesma

nucleus, and nucleic acids. Additionally, plant cells have chloroplasts, with a large number of photosynthetic pigments (chlorophyll a and b) (Staehelin 2003); amyloplasts, the function of which is to store starches and other reserve substances; and chromoplasts, which contain pigments that act as both photosynthetic pigments and antioxidants, such as α- and β-carotenes, lycopene, cryptoxanthins, lutein, lycopene, and anthocyanins (water-soluble flavonoids) (Vershinin 1999). Vacuoles or tonoplasts are organelles that occupy a large part of the cell volume. They contain water, sugars, other organic and inorganic solutes, and pigments (Hall et al. 1984).

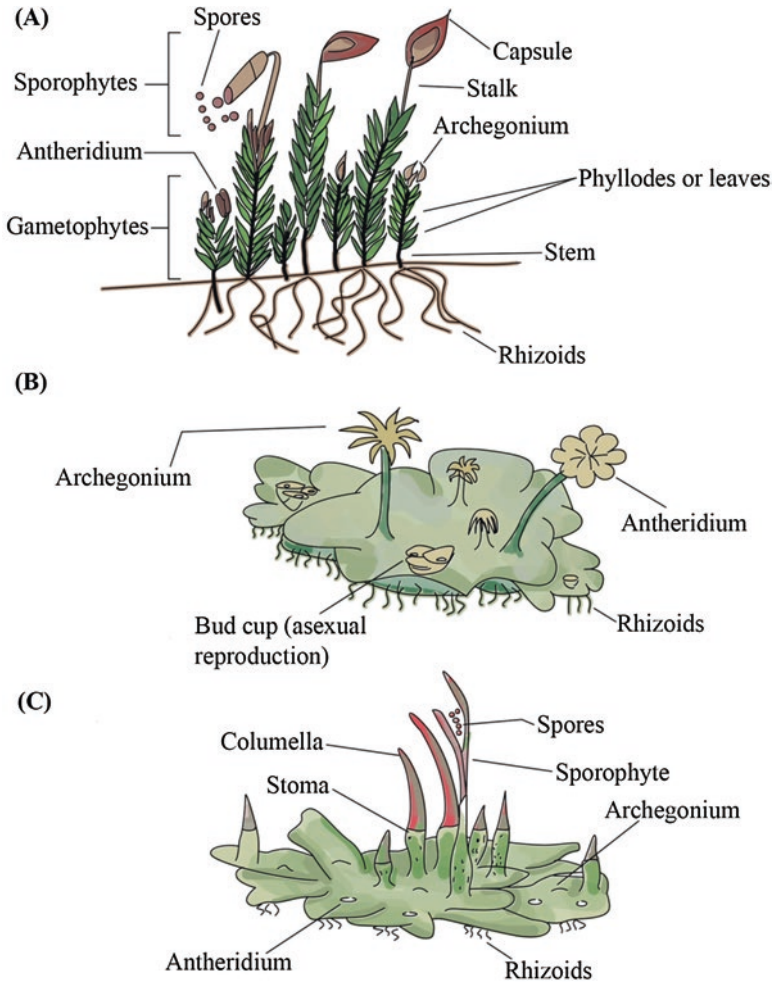

Fig. 3.2 (**a**) Main structures of a moss plant. (**b**) Liverwort plant. (**c**) Hornwort plant

3.1 Nonvascular Plants

Bryophytes (liverworts, hornworts, and mosses) (Fig. 3.2a–c) are plants that did not develop a vascular system, which is why they are known as poikilohydric plants (dependent on a layer of water on their surface to maintain their hydration). Most bryophytes can only develop in aquatic habitats, although many species are tolerant to dehydration (Zechmeister et al. 2003). Their dependence on water has been a consequence of selection pressure; therefore, these plants form simple, small, and thin tissues, and their cells lack lignin (Roberts et al. 2012). During their life cycle, they develop spores in specialized structures or sporophytes. Once bryophytes germinate, their sporophytes produce structures termed gametophores, which have elongated cells that fix the plant and absorb water from soil termed rhizoids. The thallus is the undifferentiated photosynthetic tissue that grows above the substrate and consists of epidermal and subdermal cells, thin-walled parenchymal cells, and conductive cells. The leaves, when present, usually have a thick cell wall, except in the midribs and margins, which have multiple layers of differentiated cells. Gametophytes exhibit structures that are responsible for producing gametes (sexual reproduction cells) (Fig. 3.2a–c). Bryophytes are highly sensitive to contamination; therefore, they are considered good indicators (Sheffield and Rowntree 2009; Zechmeister et al. 2003).

3.2 Vascular Plants

Vascular plants have specialized, complex tissues, including meristematic, dermal, basal, vascular, and root tissues. These plants consist of different cell lines with distinct characteristics (Fig. 3.3).

3.2.1 Tissues

The dermal tissue or epidermis is formed by a layer of cells located on the surface of the plant. It is usually covered with a waxy (lipophilic) cuticle, which protects the plants from water loss and pathogen attacks. It has cuticular pores, the diameter of which ranges from approximately 2 to 2.4 nm (Eichert and Goldbach 2008). The epidermis also has specialized cells (guard cells) that respond to variations in the external and internal environments by changing shape, opening, or closing pores or stomata (intercellular gaps up to 100 nm or greater) (Eichert and Goldbach 2008), depending on the stimulus. They are checkpoints through which water vapor, oxygen, and carbon dioxide travel. Some plant species have structures that are specialized for gas exchange termed lenticels; they lack a cuticle, and their calculated pore size is larger than 100 nm (Lendzian 2006). Trichomes are structures

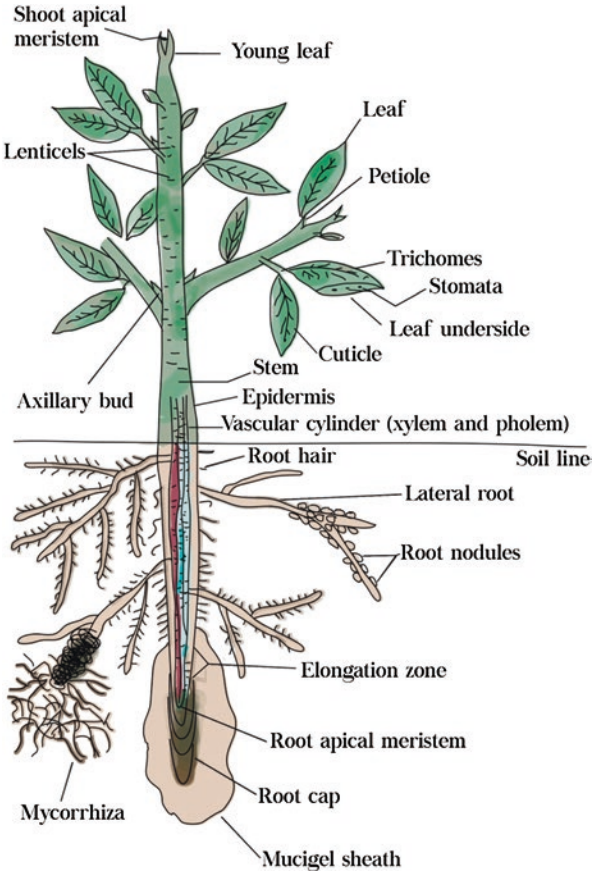

Fig. 3.3 Diagram of the main structures of a plant. Above the substrate, growth zones (shoot apical meristem) and axillary buds will give rise to branches. The stem shows lenticels, branches, and leaves, whereas the leaves have stomata. The vascular cylinder runs internally. Under the substrate, the root cap, the mucigel sheath, and the formation of lateral roots and root hairs are shown

with defensive functions in plants that accumulate heavy metals collected from the air (Fig. 3.4) (Lavid et al. 2001; Psaras et al. 2000).

Meristems are clusters of undifferentiated, thin-wall (<100 nm in thickness) stem cells (Galway 2006), which are responsible for generating cell populations that differentiate into (dermal, vascular, and growth) tissues formed at different stages of maturation. Meristems are located at growth sites in root and stem apices and in vascular cambium and cork cambium tissues (plants with secondary growth). The basal tissue mainly consists of parenchymal cells, which typically have a cell wall that varies in thickness, albeit thin, and in morphology. They are the most abundant cells in plants and are part of the mesophilic or photosynthetic (leaves and stems), epidermal, cortical (a region located between the vascular bundles and

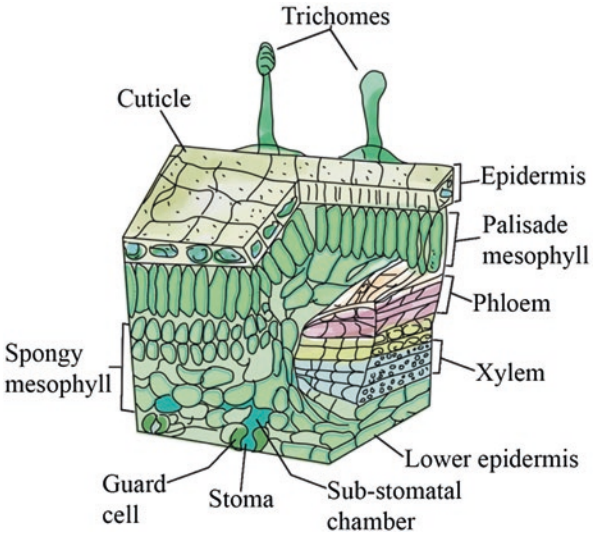

Fig. 3.4 Diagram of leaf tissues. External structures such as the cuticle and the epidermis are shown, in addition to trichomes associated with the upper epidermis. Stomata are located in the lower epidermis. Photosynthetic (palisade and spongy mesophyll) parenchyma cells and different components of the vascular cylinder are also shown

epidermis), and medullary (center of the stem) tissues and of the vascular system. Parenchyma cells specialized in storage are found in bulbs and tubers, seeds (endosperm), and cotyledons (Gibson 2012; Morris et al. 2016). In aquatic plants, these cells have a tissue termed the aerenchyma, which is characterized by intercellular spaces containing air (a specialized tissue for plant buoyancy) (Smirnoff and Crawford 1983). They can also be part of the glandular, secretory, and trichome systems. Collenchyma cells are also part of the basal tissue, small, and elongated, and their primary cell walls have different thicknesses and proportions of cellulose, hemicellulose, and pectin. These components allow them to be more elastic than other cells. They provide support to leaves and stems and are usually found near the surface of the cortex and around vascular junctions in petiolate leaves and stems (Leroux 2012). Sclerenchyma cells (fibers and sclereids) are short cells with a thick, densely lignified, and rigid secondary cell wall. They provide mechanical support to the plant. They are found next to vascular ducts and in leaf veins and margins (Fig. 3.5a) (Calvin 1967).

The evolution of lignified structures in the form of tubules, which transport water and nutrients as components of the xylem and phloem in vascular systems, has contributed to the diversification of plants with roots, stems, and leaves. The xylem is the system responsible for transporting water and dissolved minerals, consisting of tracheids and vessel elements (Fig. 3.5b). Both structures are generated by living cells, which die at maturity, leaving their thick, lignified, and interconnected cell

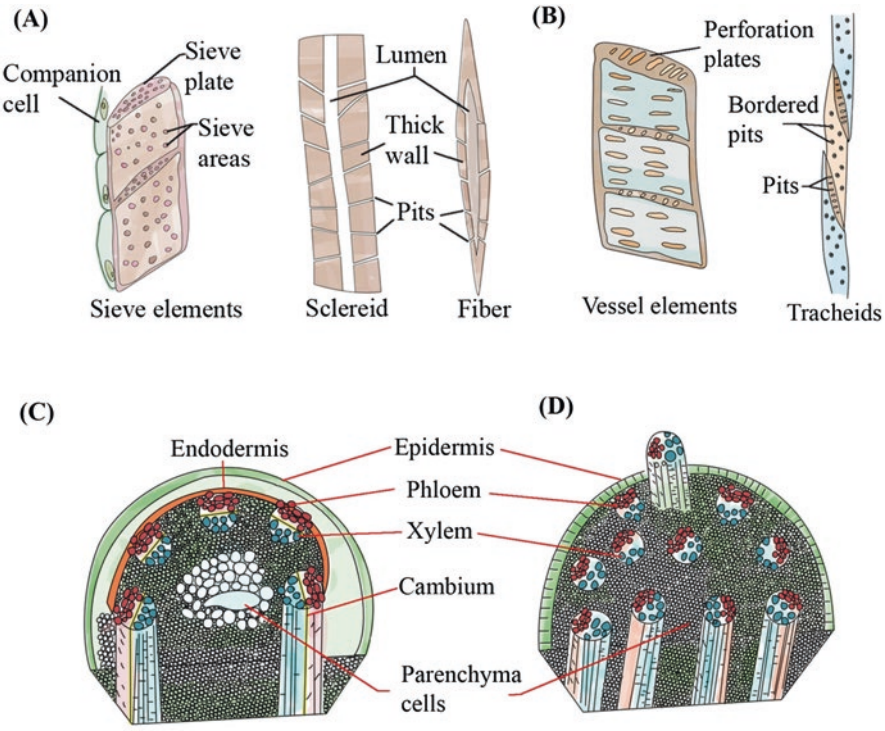

Fig. 3.5 Diagram of the main cellular structures forming the vascular cylinder. (**a**) Xylem elements. (**b**) Phloem elements. (**c**) Vascular elements in dicotyledonous plants. (**d**) Vascular elements in monocotyledonous plants

walls behind. Collectively, they form conductive tubules, the cell walls of which usually have thin areas termed pits, with an average pore diameter ranging from 5 to 420 nm in angiosperms and from 10 nm to 200 μm in gymnosperms (Fig. 3.5c) (Carlquist and Schneider 2002; Jansen et al. 2009). Liquid flows through these pits inward from the tissues or outward to the tissues. Tubule architectures and pore sizes vary between species (Choat et al. 2008). Tracheids are aligned side by side, whereas vessel elements are aligned end to end; therefore, substances in the latter are transported vertically (Luo et al. 2019). Conversely, the phloem, which functions in the transport of sugars and solutes, consists of tubular filtration elements (sieve elements) formed by living cells that are interconnected through lateral and terminal openings in their cell walls (sieve plates). Sieve elements are surrounded by specialized parenchymal cells, termed companion cells, which are responsible for transporting sugars to conductive tubes through plasmodesmata (Fig. 3.5b) (White 2012). In conifers and primitive vascular plants, sieve cells are found in the phloem. In vascular plants, the xylem and phloem are arranged in long, continuous strands, forming vascular bundles with arrangement patterns that are genetically

determined and that differ significantly among monocots, dicots, and other groups (Figs. 3.5c, d) (White 2012).

Roots usually grow below the surface; their function is to absorb water and minerals, to store nutrients, and to fix the plant to the soil. They have an apical meristem with a root cap, which is responsible for protecting the apex against mechanical damage and against the action of heavy metals present in the soil. In this region of the plant, cells secrete mucilage and exudates, composed of polysaccharides, organic acids, alcohols, secondary metabolites, antimicrobial proteins, and extracellular DNA (Driouich et al. 2013), thereby varying the microenvironmental conditions near the root tissue and promoting nutrient availability through their dissolution by changing the pH and substrate moisture (Baetz and Martinoia 2014). Morphologically, from outside to inside, the layers of root cells are arranged from the epidermis (protection), through the cortex (storage), to the endodermis formed by one or several layers of suberin-coated cells, known as Casparian strips, which function as a hydrophobic barrier (Chen et al. 2011; Lynch 1995). The pericycle consists of parenchyma cells that surround the vascular cylinder (xylem, phloem). In each organism, the root growth pattern is genetically determined. Many mono- and dicotyledonous plants have a primary root, secondary roots, and absorbing root hairs (lateral extensions), and some may also develop adventitious or aerial root systems (Longstreth and Borkhsenious 2000) (Fig. 3.6a–c). Water is transported from the roots through two pathways, the symplastic and apoplastic pathways; in both, water is absorbed through root hairs. In the symplastic pathway, water and minerals are transported from cell to cell through the cytoplasm between plasmodesmata. In the apoplastic pathway, water moves through extracellular spaces located between the plasmalemma and cell walls, albeit only up to the endodermis, wherein Casparian strips force water to enter the cells to reach the vascular cylinders (Fig. 3.6d) (Sevilem et al. 2013).

3.2.2 Seeds

Seeds contain a mature embryo, food reserves, and a coat or testa (Fig. 3.7a–c). Their formation begins with fertilization of the egg cell by sperm nuclei (pollen). The union gives rise to the zygote and the primary endosperm cell. The zygote initiates a series of mitotic divisions until the formation of a mature embryo with structures such as the root apex, shoot apex (epicotyl) with the first true leaves (plumule), and one or two seed leaves or cotyledons in mono- and dicotyledonous plants, respectively (Beeckman et al. 2000; Jones and Rost 1989). Dicotyledonous embryos usually absorb nutrients (starch, proteins, sugars, and lipids) from the endosperm, storing them in cotyledons (Maroder et al. 2003). In monocotyledonous plants, the embryo has no contact with the endosperm until the seed germinates and digestive enzymes are activated (Fincher 1989). The seeds are found in the fruits, the primary function of which is protection. In some cases, the fruits contribute to dispersion. They can be dehiscent, fleshy, simple, aggregate, or multiple.

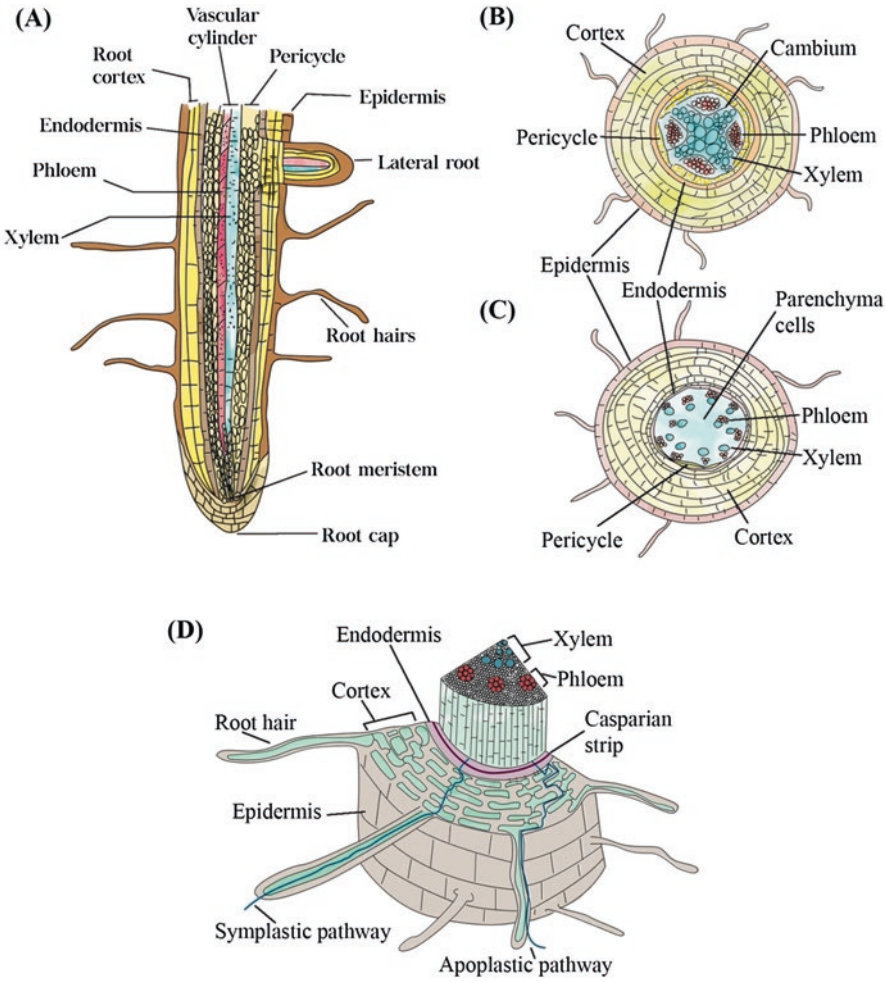

Fig. 3.6 Diagram of the tissues that form the root and vascular cylinder. (**a**) General representation of a root. (**b**) Arrangement of vascular tissues and bundles in a dicotyledonous plant. (**c**) Distribution of vascular tissues and bundles in a monocotyledonous plant. (**d**) Water and nutrient transport pathways. In the apoplastic pathway, water is transported between the interstices separating the membranes and cell walls, only until reaching the endodermis, which has cells coated with suberin (hydrophobic). In the symplastic pathway, transport occurs via the cytoplasm, through plasmodesmata

3.2.3 Germination

Germination refers to the embryo development process after the latency period, which occurs when environmental conditions (temperature, humidity, amount of oxygen, light, and nutrients) are adequate. It begins with the movement of water

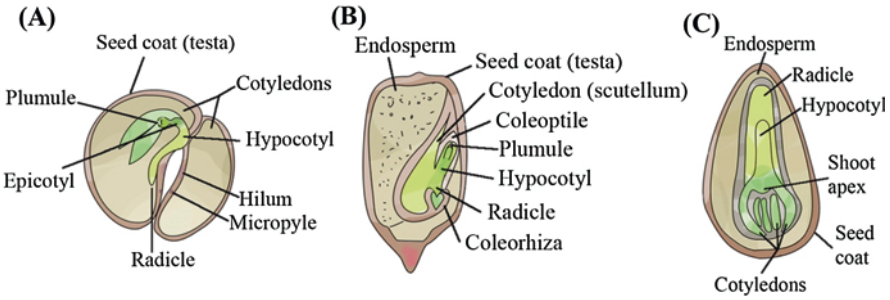

Fig. 3.7 (**a**) Diagram of embryonic structures in seeds of vascular plants. (**b**) Angiosperms. (**c**) Gymnosperms

molecules inside the seed (imbibition) by attracting the hydrophobic groups of proteins in the endosperm or in cotyledons, which swells the seed and ruptures the testa. This process increases the amount of oxygen, the temperature, and access to light hours, thus initiating the growth process, first at the root apex or radicle and then at the stem apex or epicotyl (Rajjou et al. 2012). When the seed coat or testa no longer covers the radicle, the cotyledon or cotyledons in most seeds show an increased number of photosynthetic chloroplasts, fulfilling the feeding function until the true leaves develop (Yan et al. 2014).

3.3 Associations with Microorganisms

Mycorrhizae are a mutualistic association between a plant species (pteridophytes, gymnosperms, and angiosperms) and a fungal species (*Basidiomycota*, *Ascomycota*, and *Glomeromycota*) (Martin et al. 2016; Tedersoo et al. 2010). Although they are not a plant tissue, fungi and their associations with the plant are an important element in the absorption of water and nutrients that are difficult to obtain, such as phosphates, zinc, and molybdenum, among others. The mycelium covers root surfaces and redirects them toward nutritionally rich areas, allowing the plant to take advantage of resources and grow, especially in poor soils. There are two types of plant-fungus interactions. In ectomycorrhiza, the hyphae do not penetrate the root cells but instead are located in the intercellular space (Martin et al. 2016); in endomycorrhiza, the hyphae penetrate root cells, wherein vesicles and arbuscules are formed for exchange between the fungus and the host plant (Luginbuehl and Oldroyd 2017). In turn, some plant species may also form a symbiosis with bacteria in root nodules. This association begins when nitrogen-fixing symbiotic bacteria enter roots through root hairs and subsequently infect cortical cells, wherein the bacteria reproduce and induce genetic-morphological changes. These changes lead to the formation of root nodules or tumor growths, consisting of the infected cells and plant tissue, thus enabling the plant to obtain the nitrogen necessary for its metabolic functions (Fig. 3.3) (Frank et al. 2017; Santoyo et al. 2016).

3.4 Plant Stress Response Mechanisms

Plants are continually exposed to stressors, such as variations in water availability (water stress), drastic changes in temperature, intense radiation, nutrient deficiencies (macronutrients, N, P, K, Ca, Mg, and S; micronutrients, Fe, Zn, Mn, Cu, B, and Mo, among others) (Lynch et al. 2012), overexposure to heavy metals or minerals, and pathogen infections (bacteria, fungi, worms), among others (Zhu 2016). Such factors are capable of inducing changes in physiological and genetic responses, either transient or permanent, at a local or systemic level, altering the standard conditions of metabolic functioning and growth. The main plant response mechanism includes the generation of reactive oxygen species (ROS), which involves the partial reduction of molecular oxygen (O_2) to other molecules, such as oxygen singlet ($^1O_2$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), or hydroxyl radicals (OH^-). ROS production remains at baseline levels, thanks to antioxidant systems such as catalase (CAT), peroxidase (PER), peroxiredoxin (PRX), glutathione peroxidase (GPX), superoxide dismutase (SOD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR) enzymes, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX)-like (respiratory burst oxidase homologs – RBOHs) proteins, among others; in addition, regulatory mechanisms of iron storage and uptake are also involved in maintaining ROS at baseline levels (Mittler 2017). Chloroplasts are the organelles that produce the greatest amount of ROS. ROS are also produced in mitochondria, peroxisomes, and other cellular compartments with proteins and molecules with reduction-oxidation (REDOX) reaction potential (Zhu 2016). ROS are considered signaling molecules that regulate development and differentiation near the baseline state (Noctor et al. 2018). Biotic and abiotic stress signals and interactions with microorganisms increase or decrease the presence of ROS, which can induce cytostatic or even cytotoxic effects on the plant. Exacerbated ROS production in cells can trigger chain reactions that are harmful to constitutive organic molecules. For example, oxygen radicals induce lipid peroxidation (Ayala et al. 2014; Cherchi et al. 2011; Nair and Chung 2014) and can promote enzymatic inactivation and mutations or even degradation of nucleic acids. Hypermethylation and gene expression changes are other mechanisms of plant cell defense against stress (Ghosh et al. 2019).

4 Plant Nanotoxicology

There is increasing concern about nanotoxicity in plants because they are not only a fundamental part of the ecology and ecological balance of the planet but also the basis of trophic systems. Both positive and negative effects of nanomaterials on plants have been described. Such effects vary with plant morphology, physiology, species, and the plant's uptake capacity at different stages of maturation and with the presence of mycorrhizae or root nodules. The effects of a nanomaterial are

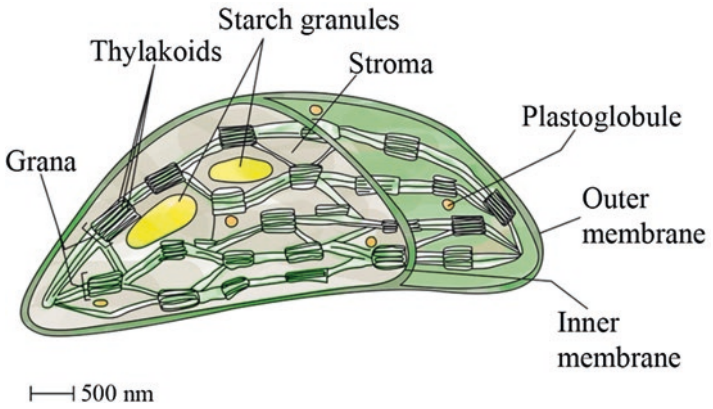

Fig. 3.8 Diagram of the sizes of structures such as chloroplasts

determined by its chemical composition, morphology, size, structure, solubility, concentration, area, and surface charge. In turn, the composition of the environment (organic matter, types of grain, pH, and humidity) and climatic conditions also help to strengthen or mitigate these effects. Characteristics such as the size, charge, and concentration of nanomaterials have been reported as the main factors underlying the uptake and distribution in plant tissues (Fig. 3.8).

4.1 Uptake of Nanomaterials in Plant Tissues

As mentioned above, nanomaterials are distributed by air, water, and land. Their mobility and toxicity vary with physicochemical factors of the particles and with their interaction with environmental components. Plants are very diverse, have colonized terrestrial and aquatic environments, and exhibit abilities to take up, resist, and respond to the presence of nanomaterials that depend on complex interactions. The uptake of nanomaterials present in the soil occurs through the root system, in which the first key interaction is with symbiotic associations. Mycorrhizal and bacterial associations can function as remediation mechanisms for toxicity induced by nanomaterials present in the soil through their retention in hyphae and nodules, thereby preventing their accumulation in roots or their translocation to other tissues and mitigating their toxic effects on host plants. Feng et al. (2013) measured the effects of silver (Ag NPs) and iron monoxide nanoparticles (FeO NPs) on mycorrhizal fungi associated with white clover (*Trifolium repens*). Simultaneously, they measured the effects of NPs on plants with mycorrhizae and in control plants without mycorrhizae. Their results revealed a decrease in fungal biomass. They also observed reduced nutrient uptake in control plants than in those with mycorrhizae.

Wang et al. (2016) also described adverse effects on maize plants and on mycorrhizae exposed to high concentrations of zinc oxide (ZnO) nanoparticles.

They observed that plants with arbuscular mycorrhizae achieved better alleviation due to the lower bioavailability of nanoparticles in the medium and therefore lower accumulation in plants. Judy et al. (2015) showed that silver sulfide (Ag_2S) nanoparticles were less toxic to tomato plants (*Lycopersicum esculentum*) with mycorrhizae than to those without, despite the decrease in fungal mass. In addition to the decrease in phytotoxicity in tomato plants (*L. esculentum*) with mycorrhizae exposed to Ag NPs, Noori et al. (2017) observed dose-dependent changes in both mycelial length and the expression of genes encoding membrane proteins (aquaporin channels, plasma membrane intrinsic protein, tonoplast membrane intrinsic channel, and potassium channels). These phenomena may have been involved in nanoparticle uptake, which was lower in plants with mycorrhizae. Associations with bacteria could have a similar effect because studies have shown that plants with root nodules exhibit some tolerance to the toxic effects of heavy metals. However, studies aimed at assessing the effect of nanomaterials have reported no results for control plants without root nodules (Cherchi et al. 2011; Guo and Chi 2014); therefore, no experimental evidence is currently available for tolerance mechanisms resulting from these associations (Tian et al. 2019). A key protective barrier, which decreases or favors the uptake of nanomaterials present in the soil, is the mucilage layer secreted by the root cap. Various researchers, such as Ma et al. (2011), have reported that the mucilage content of some plant species is able to acidify the soil, promoting the partial dissolution of some types of nanoparticles, whereas organic ligands associated with the root are able to take up metal ions. These authors showed that organic acids secreted by the cucumber plant (*Cucumis sativus* L.) modify the shape and size of the nanomaterials used (LaO_3 NPs), altering their mobility and ability to translocate into tissues. In a study of cucumber plants and 7 and 25 nm cerium nanoparticles, Zhang et al. (2011) observed that only a fraction of the 7 nm cerium NPs was able to enter the vascular system of the plant. The remaining nanoparticles were recovered in root washings, suggesting that the secreted mucilage layer functioned as a trap. The charge of the nanomaterials favors or decreases their ability to interact with plant tissues and therefore their uptake. Some studies have shown that positively charged nanoparticles are translocated at a higher rate than negatively charged materials and quantum dots. Wang et al. (2014) observed that CdSe/CdZn quantum dots coated with cationic polymers are more easily taken up by Eastern cottonwood trees (*Populus deltoides*) than QDs coated with anionic polymers. A study by Al-Salim et al. (2011) showed that quantum dots are translocated into study plant tissues of ryegrass (*Lolium perenne*), onion (*Allium cepa*), and chrysanthemum (*Chrysanthemum* sp.), possibly due to their physicochemical characteristics, again demonstrating that the charge of the particle and their interactions with plant fluids determine the mobility of QDs. Conversely, the entry of nanomaterials has been suggested to be favored in areas associated with growth, such as areas of rapid mitotic division in root meristems or in thin areas of nutrient uptake in root hairs (Fellows et al. 2003; Lv et al. 2015; Zhang et al. 2011). After entering the plant cells, the materials can move through apoplastic and/or symplastic pathways and reach the vascular ducts (Larue et al. 2012; Lv et al. 2015). The uptake

of nanomaterials in shoots (stems and leaves) is associated with the number of nanomaterials present in the air (aerosols) and with their physicochemical characteristics (hydrophobicity or hydrophilicity), allowing them to interact with organic molecules of the plant and thereby cross biological barriers such as the cuticle or induce their passage through stomata to reach the vascular system (Uzu et al. 2010). Although the mechanism of nanomaterial penetration in seeds is in turn not well known, they may be able to enter through intercellular spaces between parenchymal cells during the imbibition process, crossing the cell membrane in the endosperm (Thuesombat et al. 2014).

4.2 Toxic Effects of Nanomaterials on Plants

Several studies have shown that in plants, nanomaterials are taken up by various tissues, both roots and shoots. These nanomaterials can produce dose-dependent abiotic stresses; that is, most nanomaterials cause cytotoxicity when they reach critical concentrations, and an imbalance in ROS production is induced. Furthermore, depending on the cellular compartment and on the generated physiological response mechanisms, the condition may be local or systemic (affecting morphology, physiology, metabolism, and genetics). Some authors have suggested that the reduction in the photosynthetic capacity of plants is likely associated with lipid peroxidation in chloroplast membranes, which is associated with exacerbated ROS production in these organelles. Damage to photosystems directly results in decreased biomass (Dewez et al. 2018). In turn, changes in the numbers of carotene pigments and in phenols and flavonoids have been observed in plants exposed to high concentrations of silver nanoparticles (Gupta et al. 2018; Nair and Chung 2014). Dose-dependent genetic expression changes (upstream or downstream) have also been observed, especially in genes associated with ROS responses and those encoding cationic transporters associated with uptake (Taylor et al. 2014). Tables 3.1, 3.2, 3.3, 3.4, 3.5, and 3.6 show some damage-response examples that have been reported for vascular and nonvascular plants.

5 Nanotoxicological Evaluation Techniques in Plants

Many techniques have been used to evaluate the toxicity of nanomaterials in plants. These techniques mainly depend on studies of the nanomaterial traced within the tissues, the plant species, and the tissue type. To determine the phytotoxicity of a nanomaterial, physicochemical characterization of the nanomaterial should be performed to collect data on morphology, size, charge, surface area, and the presence of functional groups, among other properties. To evaluate morphology and size, images are usually acquired using the following high-resolution techniques: transmission electron microscopy, scanning electron microscopy, scanning transmission

Table 3.1 Nanotoxicological effects reported in mosses (nonvascular bryophytes)

Plant species	Nanomaterials (NMTs)/size/morphology	Concentrations	Stress response against NMTs	References
Physcomitrella patens	Fe NPs/20–80 nm/spherical	5, 50, 500 ng, 5, 50 µg/plant	Dose-dependent. Increase on metabolic rate and ATP production. A significant increase in ROS production	Canivet et al. (2015)
Leucobryum glaucum	GO nanosheets/8 nm/single layer	0, 0.04, 0.4, 1, 2, 4 mg/mL	Dose-dependent at concentrations greater than 1 mg/mL. Increase in ROS production. Decrease in glutathione (GSH), catalase, fresh weight, chlorophyll b, and photosynthesis. Increase in water retention. Structural cell changes (vesicle formation, cell shrinkage) and micropore formation on the leaf surface	Lin et al. (2017)
Physcomitrella patens	MnO NPs/40–50 nm and clusters 289.69 ± 73.75 nm diameter/spherical to cubic	5, 10, 20 µg/mL	Dose-dependent. High uptake, internalization, adsorption, and dissolution rates of NMTs. Increase in cell membrane permeabilization and ROS production. Gametophores with 20 mg/mL, suffered induction of clastogenic processes (DNA chain breakage and hypomethylation), and decrease in metabolic activity	Ghosh et al. (2019)
Physcomitrella patens	Ag NPs control, Ag NPs-PVP (polyN-vinyl-2-pyrrolidone), Ag NPs-citrate/37.4 ± 13.4, 29 ± 6.3, 21.5 ± 4.2, respectively/semispherical	0, 2, 5, 10 µg/mL	Dose-dependent. Response influenced by NMT coatings and plant growth stage. Using uncoated NPs in the early stage (protonema), decreased the quantities of thylakoids in the chloroplasts, chlorophyll b, and photosynthesis rate. In the gametophyte stage, the amounts of fresh and dry weight and chlorophyll b decreased	Liang et al. (2018)

Table 3.2 Nanotoxicological effects reported in seedless vascular plants (ferns) and gymnosperms

Plant species	Nanomaterials (NMTs)/size/ morphology	Concentrations	Stress response against NMTs	References
Salvinia natans (*fern*)	ZnO NPs/25 nm/ semispherical	1, 10, 20, 50 mg/L	Approximately 40% chlorophyll reduction. Reduced quantities of chlorophyll b and carotenoids. Increase in enzymatic activity of SOD, CAT, and POD	Hu et al. (2014)
Scots pine (*Pinus* sp.) Oak (*Quercus* sp.) (*gymnosperms*)	Ag NPs; Cu NPs/5 nm/not specified	0, 5, 25, 50 ppm	Dose-dependent Ag NPs: Shortening of stems and low root mass Cu NPs: Root diameter and stem length reduced With both types of NMTs (50 ppm), there were changes in the ultrastructure of needles and leaves, especially in the photosynthetic apparatus; increased quantity of plastoglobules and change in their morphology. Reduction in chlorophyll fluorescence parameters	Aleksandrowicz-Trzcińska et al. (2019)
Pinus sylvestris (*gymnosperm*)	Ag NPs/100 nm/ semispherical	0, 10, 20, 40, 80, and 100 mg/kg soil and 0, 10, 20 mg/L water	At concentrations greater than 80 mg/kg soil and 10 mg/L water. Reduction of germination percentage, root growth speed, and elongation. Reduced amount of plant fresh weight	Bayramzadeh et al. (2019)

electron microscopy, and atomic force microscopy. Spectroscopic techniques such as Raman spectroscopy, Fourier transform infrared spectroscopy, ultraviolet-visible spectroscopy, and X-ray diffraction provide data on the chemical composition, electronic properties, and crystalline structure. Particle size is measured by dynamic light scattering. The hydrodynamic radius is determined by measuring the Z-potential (Peralta-Videa et al. 2011). Inductively coupled plasma optical emission spectroscopy (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS),

Table 3.3 Nanotoxicological effects reported in vascular plants, monocotyledonous angiosperms

Plant species	Nanomaterials (NMTs)/size/ morphology	Concentrations	Stress response against NMTs	References
Oryza sativa L.	Multiwalled carbon nanotubes/diameter 10–30 nm, length 5–15 μm	0, 10, 20 40, 80 mg/L	Dose- and time-dependent. Changes in cellular morphology, chromatin condensation, and cell wall damage. Cell death by apoptosis due to bioaccumulation. Cell death by necrosis in cells exposed to initial high NMT concentrations. Significant increase in ROS production	Tan et al. (2009)
Triticum aestivum	Commercial Ag NPs and manufactured/10 and from 7.4 to 60.8/ semispherical	0–5 mg/kg sand	Dose-dependent. Growth reduction in shoot stems and roots. Increased proliferation of lateral roots. Plant biomass reduction. Increased ROS production and oxidized glutathione amount. Augmented metallothionein gene expression	Dimkpa et al. (2013)
Oryza sp. (rice)	SWCNTs, MWCNTs, fullerene C_{60}, graphene single sheets/1.1 diameter, 0.5–100 μm length, 0.5–200 μm length	50 μg/mL medium	Both single-walled and multiwalled nanotubes had a positive effect on seed germination, with an increased water uptake rate Negative effects with graphene nanosheets due to a reduction of seed germination percentage and low water uptake rate	Nair et al. (2012)
Spirodela polyrhiza	ZnO NPs/25 nm/ semispherical	0, 1, 10, 50 mg/L	Negative effects were recorded with 50 mg/L. NPs were aggregated and precipitated. Reduction in the chlorophyll/pheophytin ratio. Increased enzymatic activity of SOD and CAT. Inhibition of POD enzymatic activity	Hu et al. (2013)

and single-particle inductively coupled plasma mass spectrometry (SP-ICP-MS) are used to quantify metal nanomaterials in suspension (Bao et al. 2016; Larue et al. 2012). Some of these techniques have been useful for collecting data on nanomaterials in tissues.

Currently, there is no consensus on the optimal method for evaluating toxicity in plants, which has led to various sample preparation techniques and study parameters. Growth effects are determined by measuring root elongation and development

Table 3.4 *Continuation*

Plant species	Nanomaterials (NMTs)/size/ morphology	Concentrations	Stress response against NMTs	References
Oryza sativa L.	Ag NPs/20 nm/ spherical	0, 0.2, 0.5 y 1 mg/L	Dose-dependent. Root length reduction. Decreased weight, chlorophyll, carotenoid, and sugar content on shoots and roots. Increased ROS production, lipid peroxidation, and proline quantities. Augmented expression of genes related to oxidative stress tolerance. Reduction in mitochondrial membrane potential of roots	Nair and Chung (2014)
Lemna gibba	Ag NPs/50 nm/ spherical	0, 0.01, 0.1, 1 mg/L	Toxic effects observed with 1 mg/mL Reduction in chlorophyll synthesis. Deterioration in photosynthetic activity due to a deficiency in energy transfer. Decreased plant biomass content	Dewez et al. (2018)
Zea mays L.	La_2O_3 NPS/80–100 nm/not specified	0, 5, 50 mg/L	Dose-dependent. Acceleration of the development of apoplastic barriers. Augmented expression of genes related to lignin synthesis. Increase in the concentration of ABA in roots. Decrease in stomatic activity, photosynthetic activity, and transpiration rate. Decrease in water uptake rate. Plant growth inhibition	Yue et al. (2019)

(Lahiani et al. 2015), growth from the base of the plant to its highest point, stem diameter, number of secondary shoots, shoot length, and frond changes (Hu et al. 2013; Lee et al. 2010). Germination times, percentages, and seed viability have also been evaluated (Vannini et al. 2014). Parameters that have been useful in determining the REDOX potential by evaluating ROS production in plant tissues are the activity of antioxidant enzymes, such as catalase, ascorbic acid peroxidase, superoxide dismutase, and peroxidase, and the reduced/oxidized glutathione ratio (Dimkpa et al. 2013; Hu et al. 2013). Dye tests are also used to quantify the REDOX potential, including Alamar blue (Ong et al. 2014), nitro-tetrazolium blue (Speranza et al. 2013), and 2′,7′-dichlorodihydrofluorescein diacetate assays (Yan and Chen 2019). Conversely, X-ray absorption spectroscopy (XAS) has been very useful for locating nanomaterials in different tissue sections. Micro-X-ray fluorescence analysis (μ-XRF) and micro-X-ray absorption near-edge spectroscopy (μ-XANES) have been used to highlight the location and type of nanomaterials and to track fluorescence or measure radioactivity (Hernandez-Viezcas et al. 2013; López-Moreno et al. 2010; Lv et al. 2015; Zhang et al. 2011). Extraction with organic solvents, quantification of chlorophyll and other pigments, and relative quantification of the plant biomass (wet and dry weight) have also been used as nanotoxicity

Table 3.5 Nanotoxicological effects reported in vascular plants, dicotyledonous angiosperms

Plant species	Nanomaterials (NMTs)/size/ morphology	Concentrations	Stress response against NMTs	References
Arabidopsis thaliana	AL_2O_3, SiO_2, FeO_4, and ZnO NPs/150, 42.8, <50, and 45 nm/ semispherical	400, 2000, 4000 mg/L	Al_2O_3 NPs did not affect the germination percentage, root elongation, or number of leaves SiO_2 NPs had no effect on germination but decreased root elongation and the number of leaves at 2000 mg/L Fe_3O_4 NPs decreased root elongation, without affecting the other parameters ZnO NPs significantly decreased the percentage of germination, root elongation, and number of leaves in all concentrations tested	Lee et al. (2010)
Camellia japonica	C_{60}/C_{70} and higher fullerenes mix (79:20:1%); C60 99%	2 mg/mL	With the mixture of fullerenes, there was a decrease in the percentage of germination up to 80%. No modification with C_{60} was observed	Aoyagi and Ugwu (2011)
Cucumis sativus	LaO_3 NPs/22 nm/ spherical	0, 100, 1000, 2000 mg/L	Dose-dependent. Decrease in the size and diameter of the root. Development of greater amounts of lateral shoots. Bio-transformation of the NPs from a spherical morphology to a needle shape. Negative ultrastructural cell changes	Ma et al. (2011)
Arabidopsis thaliana	CdSe/ZnS quantum dots/ length 12, diameter 6.3/rod	5.8 nM (5 μg/ mL of $Cd2^+$)	Increase in ROS production The GSH/GSSG ratio decreased in exposed plants	Navarro et al. (2012)

evaluation parameters (Dewez et al. 2018). Finally, some studies have utilized genomic analysis (RT-PCR, endpoint PCR, qPCR, random amplification of polymorphic DNA (RAPD), and DNA microarrays) (Hu et al. 2017; Taylor et al. 2014). Analyses of DNA methylation patterns, proteomics (Vannini et al. 2014; Mustafa et al. 2015), and metabolomics have also been performed to highlight changes. Techniques such as terminal deoxynucleotidyl transferase dUTP nick end labeling (Kumar et al. 2017), the Comet (also known as single-cell gel electrophoresis) (Cvjetko et al. 2018), and Allium (Liman et al. 2019) assays have been very useful in evaluating the genotoxicity of nanomaterials.

Table 3.6 *Continuation*

Plant species	Nanomaterials (NMTs)/size/ morphology	Concentrations	Stress response against NMTs	References
Glycine max L.	Ag NPs/2, 15, 50–80 nm/not specified	0.2, 2, 20 ppm	With 15 nm NPs, 2 ppm induced decreased expression of related proteins in cellular organization (annexins and myosins). Affectation of abundances of proteins associated with stress, signaling, and cellular metabolism. 20 ppm were lethal for seeds	Mustafa et al. (2015)
Citrus maxima	g-FeO3 NPs/20 +/− 2.7 nm/ spherical	20–100 mg/L	Dose-dependent. Toxicity with higher amounts of NPs than 100 mg. Decrease in plant biomass, root length, and chlorophyll contents. Increase in the concentration of MDA produced by lipid peroxidation. Less expression in iron reductase	Hu et al. (2017)
Solanum lycopersicum	TiO_2 NPs/30–50 nm/ cylindrical/ tetragonal	0.5, 1, 2, 4 g/L	Effects observed with the concentration of 4 g/L. Decrease in seed germination percentage, plant biomass, chlorophyll content, and photosynthetic efficiency. Increase in enzymatic activity of CAT and PXD in roots and leaves and in the expression of glutathione s-transferase and glutathione synthase. Alteration in the transport rates of essential elements (P, S, Mg, and Fe)	Tiwari et al. (2017)

6 Conclusions

Nanotechnology is a discipline that has acquired great relevance in many areas of research (physics, chemistry, biology, engineering, electronics, medicine, etc.), as well as in industrial and pharmaceutical development. A perspective of nanotechnology is the expansion of promising applications, such as antimicrobial bandages, drug carriers, catalysts, scratch-resistant coatings, self-cleaning glasses, semiconductors, and UV-protected garments. However, as with many technological developments, nanotechnology may have both positive and negative effects in terms of health and the environment.

In the last decade, the number of nanomaterials included in objects and devices of common use has increased. However, the possible environmental consequences, resulting from the massive use of nanomaterials, have not been thoroughly addressed. Materials at the nanoscale behave differently than they do in their bulk

form and may be a risk to living organisms at different organization levels. Nanotoxicology is a relatively new research area which addresses this issue by studying the uptake, accumulation, chemical interaction, and biological effects of nanomaterials. Novel methodologies are being developed to characterize the nanomaterials present in the environment and to better understand their interaction with cells and tissues, in order to determine if they constitute a threat. Soil microorganisms in symbiotic association with plants are the first link damaged by an increase of nanomaterials in the environment, sometimes considerably reducing the growth of plants. When nanomaterials reach plant tissues by penetrating through their openings in roots, stems, and leaves, they may induce stress mechanisms that could achieve toxic levels in tissues, causing deterioration of plants, altering whole crops or even ecosystems. Furthermore, due to a bioaccumulation phenomenon in plants, nanomaterials can enter the food chain of animals and humans constituting a health problem. Therefore, extensive safety research projects and regulations on the use of nanomaterials are still needed. In this chapter, basic concepts of plant nanotoxicology were described, including some properties of nanomaterials, and the anatomy and physiology of plants, as well as the methodologies so far existing to evaluate the toxicity of nanoparticles.

Developments using nanomaterial engineering for specific purposes should be accompanied by the corresponding toxicological studies and by studies assessing the potential environmental damage.

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Chapter 4
Carbon Nanotubes as Plant Growth Regulators: Prospects

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1 Introduction

Carbon nanotubes (CNTs) are available as single-walled carbon nanotubes (SWCNTs), double-walled carbon nanotubes (DWCNTs), triple-wall carbon nanotubes (TWCNTs), or else multiwalled carbon nanotubes (MWCNTs). These functional nanoscale materials have a variety of unique, fascinating, and never-seen-before properties. DWCNTs are coaxial nanostructures composed of exactly two SWCNTs, one nested in another. MWCNTs consist of multiple nanotubes inside larger nanotubes with the same and different chiralities.

Currently, carbon nanotubes are being used in a wide range of modern technologies to harness their properties never seen before in other materials. These particular properties could improve crop management worldwide with exceptional advantages for farmers. However, there are also innumerable health and environmental concerns regarding the side effects of nanotechnologies, particularly, when the use of nanotechnology implies the spreading of novel materials in ecosystems where CNTs had never been in touch.

It has been reported that c.a. 14,000 tons of MWCNT were synthesized in 2016, although the unregulated use of these materials could release them to the environment during production, transportation, handling, use, and final disposal (Zhao et al. 2017). However, natural substrates such as soil, water, or air become the ideal site for final disposal worldwide, particularly in countries of low incomes.

After a broad database study, it was found that there are two groups with opposing results; one group presents that CNTs are severally phytotoxic, but another group suggests that the CNTs have positive extent beneficial effects on crops. SWCNTs, DWCNTs, or MWCNTs have been widely studied by plant biologists, because of their particular effects on the growth of different crops and the promises of their potential use as smart delivery systems in plants.

The CNTs have been evaluated in plant growth chambers, greenhouses, or lands to determinate the potential effect of these materials on crops with environmental, social, or economic importance. So, this chapter summarizes the main advantages and drawbacks regarding the use of CNTs as plant growth regulators.

2 Classification of the CNTs and Their Main Characteristics

As we know, the carbon nanotubes are like a pipe of carbon formed by a sheet of graphene (monolayer). They are macromolecules of graphene that are arranged by layers or blades of interlaced molecules of carbon given a cylinder arrangement (Karimi et al. 2017), where the interlaced is a typical hexagonal. The main characteristics of the CNT are they are 100-fold more rigid and flexible than steel. The CNT has a basic unit, the hexagonal form, like graphene. Another important property, derived from its structure, is the thermal stability, maybe semiconductor depending on diameter and chirality of atoms.

2.1 Classification of the CNT According to the Wall Structure

On this classification, there are two main groups, such as SWCNT and MWCNT; on the last one, there have been included DWCNT, TWCNT, or more layers. The first one, the SWCNT, was reported in 1993. They are structured by a single layer of carbon given a cylindrical form with 0.4–2.3 nm of diameter range, where 1 nm is the most common diameter, and the longitude can be various millions of units (20–1000 nm) (Karimi et al. 2017). According to the cylindrical structure of CNT, we can find several chemical configurations. A chiral vector defines this structure; this means that the CNT axis and the hexagonal lattice orientation are related in the configuration through chiral indexes, *n* and *m* (Fig. 4.1), i.e., represent the number of unit vectors along of two directions in the crystal lattice of graphene. The single-walled carbon nanotube (SWCNT; Figs. 4.2 and 4.3) is considered a quasi-one-dimensional (1D) material starting of rolling up a graphene sheet (Okuyama et al. 2019).

2.2 Classification of the SWCNT by Carbon Configuration

The SWCNT can be classified by configuration, such as an *armchair*, *zigzag*, and *chiral*. The armchair structure is a hexagonal structure building by cyclohexene, where $n = m$, the zigzag form has $m = 0$, and chiral form is defined by angle Ø (angle between the zigzag direction and the chiral vector), so this angle is given by $\text{Ø} = \tan^{-1}\left[\frac{\sqrt{3}m}{(m+2n)}\right]$. The term chiral (means "hand" and it is provided by asymmetric

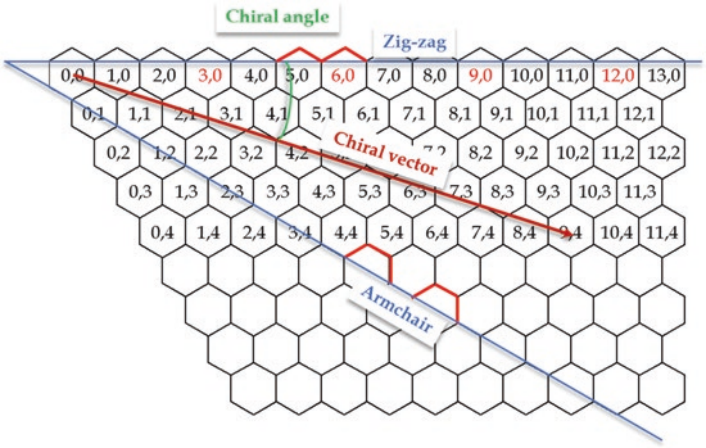

Fig. 4.1 Model of chiral vector, where the CNT axis and the hexagonal net orientation are related in the configuration of the structure of CNT

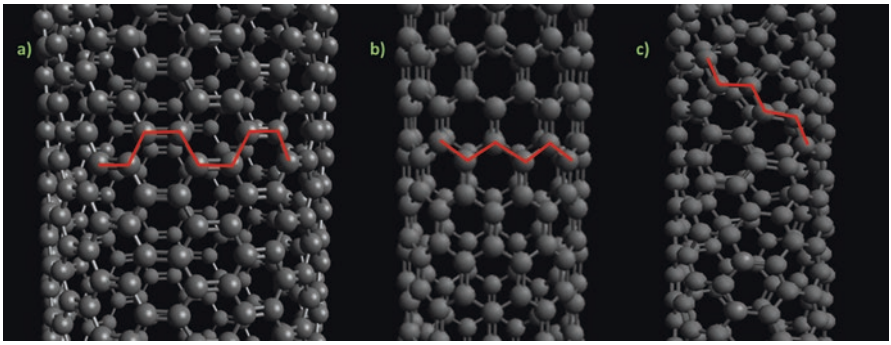

Fig. 4.2 A schematic model of SWCNT derived from the model of the chiral vector. (**a**) Armchair (10, 10), (**b**) zigzag (10, 0), and (**c**) chiral (6, 4) models. (Models were created with Avogadro software (Version: 1.2.0))

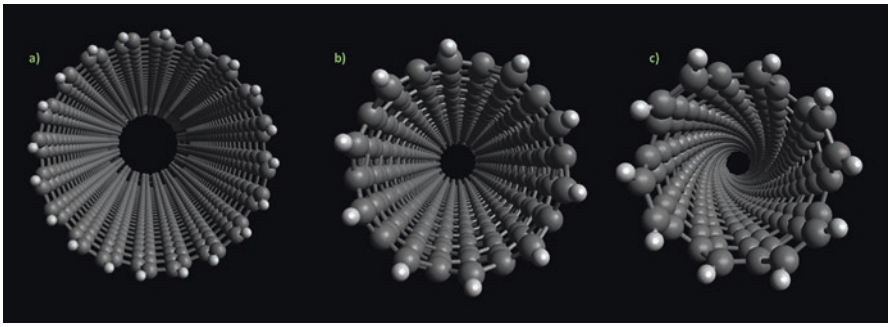

Fig. 4.3 A model of SWCNT from an inside view. (**a**) Armchair (10, 10), (**b**) zigzag (10, 0), and (**c**) chiral (6, 4) models. (Models were created with Avogadro software (Version: 1.2.0))

carbons) refers to compounds that are typically optically active, where the asymmetric structure of carbon is a mirror image that is not superimposable.

Multiwalled carbon nanotubes (MWCNTs) are formed by two, three, or more CNTs of graphene, arranged concentrically (Fig. 4.4). They can organize by a *double wall*, *triple wall,* and *multiple walls* (more than three CNTs concentrically). The double-walled carbon nanotube (DWCNT) is composed of two different cylindrical (two nanotubes of graphene) parts that have in common their axis of symmetry (coaxially). Three different cylindrical nanotubes characterize the triple-wall carbon nanotube (TWCNT) arranged concentrically as well, reaching diameters over than 10 nm. As we know, in the single-walled CNT, the carbons are united by covalent bond atom by atom; however, in the multiwalled NT, the concentric tubes are united by van der Waals interactions, forces that maintain the space between nanotubes. They have lengths between 1000 and 50,000 nm (Karimi et al. 2017).

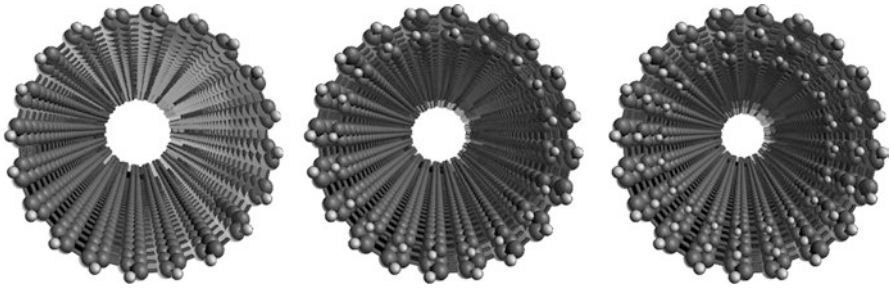

Fig. 4.4 A model of CNT from an inside view of single wall (SWCNT) and multiple walls (DWCNT and TWCNT)

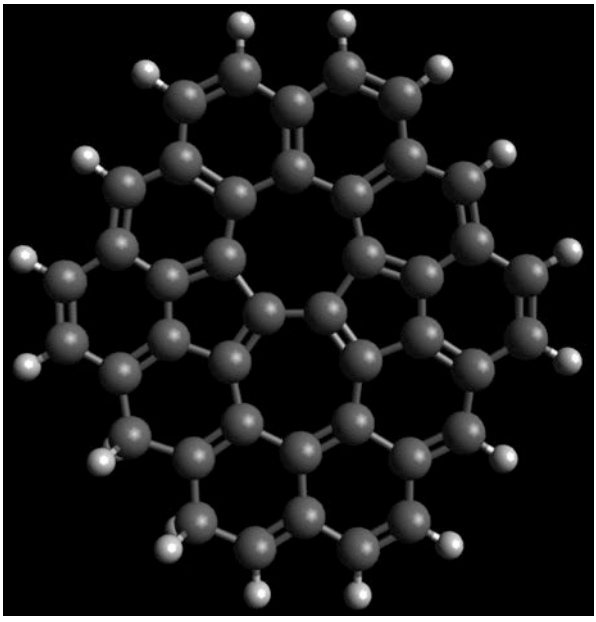

Fig. 4.5 Stone-Wales defect of CNT. This defect is called 7:5:5:7 of the sidewalls of a CNT. (The model was created with Avogadro software (Version: 1.2.0))

According to higher length and taking into account the chemical synthesis, it can be seen some kind of faults, the Stone-Wales defects, for example, the 7:5:5:7 defect that shows two rings of seven carbons and one pair of five-carbon rings as well (Fig. 4.5), deforming the curvature of the tube locally.

2.3 Characteristics

The exceptional properties of CNT are due to their chemical, physical, and physico-chemical properties such as their high surface-area-to-volume ratio, high elastic modulus, tensile strength, stronger than steel, and harder than diamond. CNT can

behave as conductors transferring heat (thermal conductivity higher than diamond) and electrical current higher than copper (Kaminskyj 2008; Mukesh and Jha 2017), and they can exhibit unusual mechanical properties as high toughness and high elastic moduli (Mukesh and Jha 2017). Besides, concerning electronic structure, they can exhibit semiconducting and metallic behavior (Mukesh and Jha 2017), allowing a wide opportunity for doing prominent research, versatile, and an efficient delivery vehicle for agricultural, food, and biomedical applications, and other advanced technological (such as electronics, effecting materials, environment, healthcare, and energy) applications as well, offering a countless potential.

3 Repository, Movement, Fate, and Bioavailability of Natural or Engineering CNTs in the Environment

Carbon nanotubes (CNTs) have different behaviors under natural conditions depending on their length, diameter, functionalization, or environment (Chen et al. 2019). CNTs are characterized by their high resistance, low density, strong hydrophobicity, and severe biodegradation. Hence their impact on the environment will depend on their ability to interact with natural organic matter (NOM) (Khosravi-Katuli et al. 2017). Although CNTs are hydrophobic in aqueous suspensions, scientists have explored different methods to increase their solubility by adding diverse chemical groups to their surface. These modifications can improve their aqueous solubility and biocompatibility; this, in turn, can increase their toxicity because these functional groups might be exchanged with NOM (Chen et al. 2019; Chang and Bouchard 2015). Unfortunately, this process is not wholly understood. Therefore, more work is needed to understand the fate and toxicity of CNTs—especially in terrestrial plants and ecosystems.

3.1 Effects and Fate of CNTs in Plants

Several studies have demonstrated that 10–200 μg/mL CNTs can stimulate seed germination and growth of model plants as well as crop species such as barley, chickpea, corn, broccoli, canola, sorghum, onion, radish, etc. (Oloumi et al. 2018; Pandey et al. 2018; Liné et al. 2017; Martínez-Ballesta et al. 2016). Fan et al. (2018) reported that CNTs could modulate the toxicity of methyl viologen by adsorbing to their surface. These protect the plant root by stimulating photosynthesis, activating the antioxidant system, and increasing the number of lateral roots that help to assimilate nutrients. However, Begum et al. (2012) recorded the phytotoxic effects as a function of CNT concentrations greater than 1000 μg/mL. This delayed seed germination, decreased shoot length, lysed cells, and consequently led to plant death. These authors hypothesized that the toxic effects might be due to the aggregation of the CNTs in the roots, which would impede water and nutrient uptake.

At the cellular level, CNTs can increase xylem and phloem cell size and stomatal density, activate aquaporins, and promote the absorption of nutrients via an increase in lateral roots (Verma et al. 2019; Joshi et al. 2018a, b; Liang et al. 2013). Other studies noted that CNTs could improve the functioning of photosynthetic machinery because CNTs can integrate into the outer lipid envelope of chloroplasts; their semiconducting capacity tripled the photosynthetic activity through an increase in electron transport (Fan et al. 2018; Giraldo et al. 2014; Calkins et al. 2013).

The cellular location of CNTs in plants is poorly understood, but it depends on the uptake pathway; potential pathways include proteins, transport vesicles, or direct internalization. The latter depends on the ratio height and the radius of the nanomaterial. CNTs cylindrical shape and length allows them to enter through the cell wall and the plasma membrane of plant cells similar to a nano-syringe (Zhai et al. 2015; Serag et al. 2013; Liu et al. 2009; Kostarelos et al. 2007).

Wong et al. (2016) noted that the passive transport of CNTs is due to their interactions with lipids as well as their size and charge. The charge of CNTs affects their movement through plant tissues and cells. Zhai et al. (2015) observed that the transport of CNTs in the vascular system occurs via transpiration through the xylem toward the leaves. Subsequently, they are translocated to different parts of the cells such as the cell wall, cell membrane, cytoplasm, chloroplast, and mitochondria depending on their charge and size (Fig. 4.6). The small size and homogeneity facil-

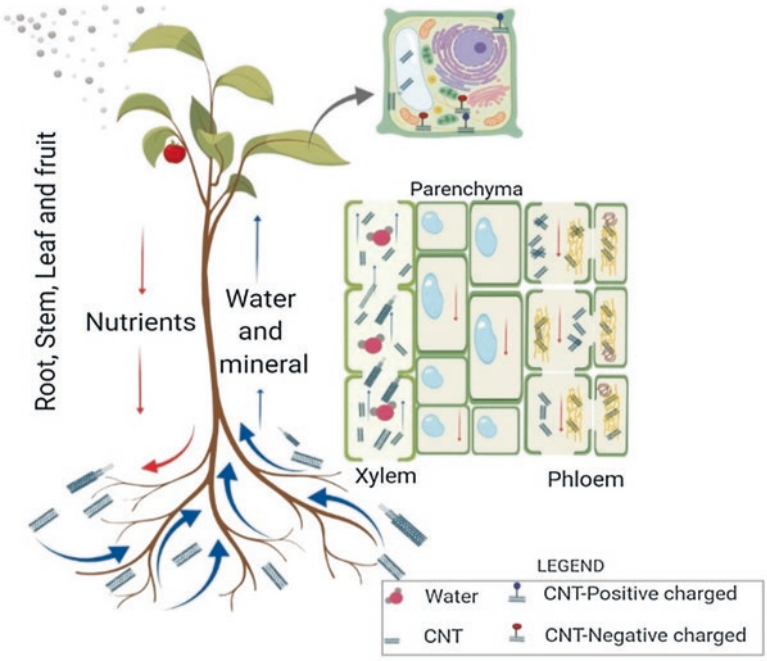

Fig. 4.6 Uptake and translocation of carbon nanotubes (CNTs) after exposure to the plant system

itates this uptake, but the charge allows the CNTs to be transported to cellular organelles: Negatively charged CNTs are better dispersed in the cytoplasm, and positively charged CNTs remained on the surface of the cell membranes and organelles (Zhai et al. 2015; Larue et al. 2012a, b). Das et al. (2018) reported that CNTs functionalized with carboxyl groups were more easily assimilated and translocated from the roots to the leaves. The CNTs that were not functionalized were transported to the vacuole; however, these same CNTS localized in the cytosol when conjugated with DNA (Serag et al. 2013; Liu et al. 2009). Thus, CNTs have generated interest regarding their use as an intelligent drug and gene delivery system in plants (Cunningham et al. 2018); their large surface area offers strong potential for chemical modifications (Kwak et al. 2019; Li et al. 2017).

Khodakovskaya et al. (2013) studied tomato plants and demonstrated that CNTs added to the substrate via irrigation water could increase the number of leaves and flowers; the CNTs also migrated to the plants' reproductive organs. The presence of CNTs in the tissues of plants can be perceived as a stress factor triggering signal transduction similar to those triggered by the attack of pathogens or herbivores. This, in turn, induces massive changes in the plant's gene expression, metabolism, and physiology (McGehee et al. 2017; Hu et al. 2015). Therefore, CNTs can alter the concentrations of phytohormones and the enzymatic and nonenzymatic antioxidant system—both of which play essential roles in counteracting the phytotoxic effects of CNTs (Hao et al. 2018). The impact of the CNTs depends on their inherent characteristics, purity, presence of amorphous carbon, agglomeration and distribution in plant tissues, chemical functionalization, and species of plant (Haghighi and da Silva 2014; Villagarcia et al. 2012).

3.2 Release of CNTs

The main use of CNTs in agriculture that can lead to their accumulation in plants is as a nanosensor for various tasks: (i) detection of pesticides (Tang et al. 2019; Bandyopadhyay et al. 2017) and secondary metabolites (Erady et al. 2019), (ii) release of pesticides and fertilizers (De La Torre-Roche et al. 2013; Sarlak et al. 2014) to increase growth and development (Bakytkarim et al. 2019), and (iii) utilization as a source of new materials to increase the life of fruits after harvest (Liu et al. 2019). Currently, there is a growing trend in the use of CNTs in commercially available products such as agrochemicals (pesticides, fertilizers) in addition to their applications in electronics, materials, and medicine. Thus, thousands of tons are produced per year (Jia and Wei 2019), leading to the release of CNTs into the environment and agricultural systems.

CNTs usually have low toxicity to soil microorganisms relative to metal nanoparticles (Simonin and Richaume 2015). However, CNTs can alter the relative abundance of bacteria and the compositional profiles of the soil bacterial community; however, the diversity and phylotype of microbial communities are not affected (Wu

Table 4.1 Degradation of carbon nanotubes by chemical and enzymatic methods

	Method of degradation	Sub-products of degradation	References
Chemical			
SWCNT, MWCNT	Sodium hypochlorite	Carbon oxides or carbonates ions	Zhang et al. (2019)
SWCNT, MWCNT	Nitric acid and sulfuric acid	CO_2, –COOH	Wang et al. (2019)
MWCNT	Electrochemical	Amorphous carbon	Reipa et al. (2018)
Enzymatic			
SWCNT	Myeloperoxidase	Small quantities of carbonaceous residues	Martín et al. (2019), Kagan et al. (2010)
SWCNT	Eosinophil peroxidase	Complete degradation	Kotchey et al. (2013), Andón et al. (2013)
SWCNT, MWCNT	Horseradish peroxidase	Morphological changes, defects on the nanotubes, and shortness	Kotchey et al. (2012), Russier et al. (2011), Zhao et al. (2011)

SWCNT single-walled carbon nanotube, *MWCNT* multiwalled carbon nanotube

et al. 2019). Some of the taxonomic genera affected by the presence of CNTs in soils are *Nitrospira*, *Planctomyces*, and *Lysobacter* (Hao et al. 2018).

The studies noted above confirm the bioaccumulation of CNTs by plants, but little is known about the bioaccumulation of CNTs. The possible transformation of CNTs in natural systems can change their properties and consequently affect their mobility and bioavailability. Some studies confirmed that CNTs could be degraded (Table 4.1) by chemical and enzymatic methods. Also, CNTs can be degraded by *Burkholderia kururiensis*, *Delftia acidovorans*, and *Stenotrophomonas maltophilia*. This process can decompose multiwalled carbon nanotubes (MWCNTs) into CO_2 and other by-products (Chen et al. 2017a, b). Importantly, these bacteria are common in the rhizospheres of soil, surface water, and groundwater. Biodegradation of CNT might be via peroxidase (DyP) type and cytochrome P450 (CYP3A4) (EL-Sayed et al. 2019; Zhang et al. 2014). The biodegradation of CNTs by microorganism and enzymes is critical to removing them and reducing their negative impacts on the environment.

4 Main Uses of CNTs in the Agriculture Sector

Modern agriculture requires a variety of agrochemicals for increasing crop yields to throw control of phytopathogens and plant diseases (Rai et al. 2015). It is well known that the uncontrolled use of high quantities of agrochemicals such as fertilizers or pesticides increases the pathogen and pest resistance, the environmental pollution, and the bioaccumulation of pesticides while reducing the diversity and abundance of microorganisms from water, air, and soil. Besides agrochemicals play

an important role regarding the risks to animal and human health (Rai et al. 2015; Solanki et al. 2015).

Several reports highlight the uses and applications of carbon nanotubes (CNTs) in agriculture as fertilizers, herbicides, pesticides, and nanosensors for monitoring soil and environmental conditions or on disease detection, among others (Aouada and De Moura 2015; Liné et al. 2017). The CNT could be found anywhere in the plant structure, as reported by Liné et al. (2017). They showed the processes involved in the uptake and distribution of CNTs in plants, such as (i) CNTs enter the plant roots by osmotic pressures, capillary forces, and pores on cell walls; (ii) CNTs cross through both cell wall and cell membrane (endocytosis); (iii) they may be transported by the vascular system with water and nutrients; (iv) they are found in leaves (mainly in the leaf xylem) and other upper parts of plants; and (v) in cell, CNTs accumulate mainly in cell wall, membrane, chloroplast, mitochondria, and cytoplasm.

4.1 Fertilizers

CNTs are applied in agricultural systems as a complement of the essential nutrients of crops; nevertheless, it is important to make most effective the use of chemical fertilizers and minimize the risks of ecological contamination (Solanki et al. 2015). The CNT as fertilizers carrier or controlled-release vectors, so-called "smart" fertilizers and they could improve the modern agriculture through new enhancements, due to the controlled fertilizer delivery such as chemicals, biofertilizers, micronutrients, and other plant growth-promoting which could be more efficiently applied on lands. So, the CNT should improve uptake efficiency, increase the plant growth and therefore a high biomass production, produce food with higher crop yields, and reduce the cost of the ecological contamination and other drawbacks (Aouada and De Moura 2015; Liné et al. 2017; Mastronardi et al. 2015; Solanki et al. 2015). So, the nanofertilizers could regulate (i) the solubility and dispersion of mineral micronutrients; (ii) the nutrient uptake efficiency to reduce the fertilizer doses; (iii) release modes; (iv) effective duration and to extend half-life of nutrients into soil; (v) loss rate of fertilizers nutrients into soil by leaching and/or leaking; (vi) release of fertilizers on-site target, reduction toxicity; and (vii) the CNT under drought conditions, can be as plant growth promoters or crop protectors (Mastronardi et al. 2015; Solanki et al. 2015) and minimize the risks of global contamination and degradation (Solanki et al. 2015).

For example, some CNT as SWCNT significantly affected root elongation of tomato, cabbage, carrot, and lettuce and promoted the growth of onion and cucumber (24–48 h); other crops showed effects on root elongation of *Allium* spp., *Brassica oleracea*, *Cucumis sativus*, *Daucus carota*, and *Lactuca sativa* (Ma et al. 2010; Subramanian et al. 2015). The MWCNT at concentrations range of 10–40 mg mL^{-1} enhanced the seed germination and growth of tomato plants (Ma et al. 2010; Khodakovskaya et al. 2009). Similar results were found for some CNT when they showed increase on seed germination and plant growth, Table 4.2 (Mastronardi et al. 2015).

Table 4.2 Beneficial effects of CNT on some crops

CNT type	Concentration (μg mL^{-1})	Crops	Effects	Findings	References
CNT	10–40	Tomato	Seed germination	Accelerate the process of seed germination which significantly shortened the germination time: 74–82% to 12 days, 90% to 20 days	Khodakovskaya et al. (2009)
			Biomass	Fresh weight of total biomass (leaves, stems, and roots), increases 2.5-fold than the control treatment	
			Length of stems and roots	Seedlings had longer stems and were more developed but showed similar lengths of a root system than the control treatment	
MWCNT	No data	Tomato	Flowers and fruits	Produce two times more flowers and fruits	Khodakovskaya et al. (2013)
MWCNT	2000	Ryegrass Rape Corn	Root elongation	1.2 better than control 1.3 better than control 1.3 better than control	Liu and Lal (2015)
MWCNT	104–1750	Onion Cucumber	Root elongation	1.8–2.8 better than control 1.4–2.0 better than control	Liu and Lal (2015)
MWCNT	50–200	Tomato	Tomato biomass seedlings and fruit number each plant	1.1–1.2 better than control 2.3–2.5 better than control	Liu and Lal (2015)
MWCNT	50, 100, 200	Barley Corn Soybean	Shoot length	1.4 better than control 1.1 better than control 1.2 better than control	Liu and Lal (2015)

(continued)

Table 4.2 (continued)

CNT type	Concentration (μg mL^{-1})	Crops	Effects	Findings	References
MWCNT	No data	Barley Corn Soybean	Root elongation	1.4 better than control 1.3 better than control 1.1 better than control	Liu and Lal (2015)
SWCNT	6.0	Chickpea	Roots, shoots, and stems	Showed increased growth rates	Tripathi et al. (2011)
SWCNT	0.16, 0.9, 5 g	Onion Cucumber	Root growth Germination seeds	Better than control	Liu and Lal (2015)

Nevertheless, some MWCNT showed phytotoxicity activity, reducing the biomass and root cap malformation of *Cucurbita pepo* (zucchini) (Ma et al. 2010). There are findings where CNT can be beneficial or deleterious effects for the same crop, or helpful for a specific crop, but deleterious for another crop.

4.2 Pesticide

Some practices for crop production include the use of new herbicides, insecticides, fungicides, etc., to avoid or reduce the crop losses regarding diseases and pests which decrease the yield from 20% to 40% (Borgatta et al. 2018). So, cutting-edge technologies with scientific advances never seen before have been proposed for innocuous and affordable food production such as biotechnology and nanotechnology. These breathtaking and latter-day knowledge areas might play an outstanding function in the production of edible or nonedible crops. CNT has a potential structure for the diffusion of antimicrobials; thus it can penetrate the plant cell walls (Liné et al. 2017), pesticides, and delivering slowly with the aim increasing agrichemicals efficiency in target crops (Brandelli 2015; Liné et al. 2017). Also, some innovations by CNT for agricultural practices include delivery of DNA or RNA and nanosensors to detention crop diseases and monitor soil (Table 4.3) (Borgatta et al. 2018). It has been well studied that the transport of DNA or RNA some times can stimulate several mechanisms to fight and defending from plant pathogen target through the activation of some resistance mechanisms, such as the onset of the enzymatic expression or the synthesis of compounds with antimicrobial activity against phytopathogens or plant diseases (Brandelli 2015). On the other hand, the agrichemicals with CNT could be applied as a new technology for genetic engineering and might work as nanosensors detecting the degradation of pesticides with catalytic or photocatalytic reactions. Therefore, the CNT can carry out and deliver DNA or RNA for monitoring the soil, ecological resilience, or increasing the efficiency of the disease detection systems (Aouada and De Moura 2015).

Table 4.3 Pesticide activities by CNT on some crops

CNT type	Concentration (μg mL^{-1})	Crops	Effects	Findings	References
MWCNT	No data	Microbial community of soil	*Bacteroidetes* and *Firmicutes*	Increase	Khodakovskaya et al. (2013)
MWCNT	No data	Microbial community of soil	*Proteobacteria* and Verrucomicrobia	Decrease	Khodakovskaya et al. (2013)

4.3 Other Concerns

The most commercial nanoparticles products need some certain stabilizers because their duration in suspension is very short, and some synergetic effects the nanoparticles and stabilizers could be phytotoxicity for certain crops (Ma et al. 2010). It is essential to regulate production and uses of nanomaterials for a safe and sustainable future. So far, there is no international agreement to supervise the production, use, and commercialization of nanomaterials (Gwenzi and Chaukura 2018).

The CNT can help the modern agriculture, and they are more environmentally friendly, can reduce chemical dosage, and can be specific and efficient, with potential to solve agricultural problems caused by conventional crop management (Rai et al. 2015).

5 Uptake, Transport, and Accumulation of CNTs in Plant Cells

Up to 2016, the global production of MWCNT was approximately 13,996 tons. It is precisely the increase in demand and the use of CNTs that could be released to the environment during production, transportation, handling, use, and disposal, being the soil the final destination (Zhao et al. 2017). The hydrophobic character of the carbon nanotubes makes them concentrate on the soil 20 times more in comparison with the concentration that can be reached in water, so that interactions with plants are even more likely, and there may be a tendency to bioaccumulate in tissues of plants and biomagnify in the trophic chain, which represents a risk to human health (Zhao et al. 2017). So far there are two groups of opposing results; one group presents that the carbon nanotubes (CNTs) are severally phytotoxic; on the other hand, other authors suggest that the CNTs have positive and to a certain extent beneficial effects on diverse physiological processes of the plants.

The positive effects of CNTs on the growth and development of plants have been documented by several research groups, having reported that increased the growth of roots in onion and cucumber plants (Cañas et al. 2008) as well as grass ryegrass (Lin and Xing 2008). Also, it has been shown that wall carbon nanotubes multiple

(MWCNT) can activate the growth of tomato plants and affect the expression of genes that are essential for cell division and plant development (Villagarcia et al. 2012). Liu et al. (2009) demonstrated that single-walled carbon nanotubes have the right size to penetrate the walls and membranes of the tobacco cells. It has generated interest because in a similar way to aquaporins, it can help transport water and nutrients very quickly inside plants (Joseph and Aluru 2008). They are also useful for desalination processes since they can be charged internally with Na^+ and Cl^- ions and then remove excess salts from the system (Goh et al. 2013). Stampoulis et al. (2009) reported negative effects such as reduction of zucchini biomass in response to the application of MWCNTs. Lin et al. (2009) found that the addition of SWCNTs to rice plants resulted in the delayed flowering and decreased the yield of this important crop.

SWCNTs, MWCNTs, and single-walled carbon nano-horns (SWCNHs) have been more studied by plant biologists, because of their particular effects on growth of different crops and the promises of their potential use as smart delivery systems in plants. A critical fact is that the metal contamination in different carbon-based nanomaterials (graphene, carbon nanotubes, fullerenes, nanodiamonds, and nanofibers) can modify the response of plant (Lahiani et al. 2015).

Currently, knowledge shows that the uptake and transport of ENPS depend on the properties of nanomaterials, for example, size, aggregation and size-dependent sedimentation or diffusion toward the cell, soil conditions, dose and method of application, and plants conditions (Lin and Xing 2008). Lee et al. (2008) found a linear relationship between high concentrations of CuNPs in the growth media and higher uptake and accumulation of CuNPs in plant tissues; this could be another influencing factor of uptake and transport. Raliya et al. (2016) reported that the uptake and translocation almost depend on the shape of ENPs, for example, if the NPs are spherical, rhombic, or cubic, among others; as a result, there is a need for a thorough understanding of the effects that carbonous nanotubes have on the physiology and development of plant systems at various levels.

The uptake and accumulation of different ENPs by plants is at present a motivation for some scientists. But this phenomenon is not common to every plant species. The uptake of nanoparticles by vegetal cells could happen in several ways, through aquaporins, binding to carrier proteins, endocytosis, ion channels, creating new channels by CNTs, or binding to organic chemicals (Fig. 4.7). This last property is a particular characteristic of carbonaceous hole nanostructures. Liu et al. (2009) reported the ability of single-walled CNTs (SWCNTs) to penetrate the walls and membranes of tobacco cells; similar results were shown for Serag et al. (2011); they identified an endosome-escaping uptake mode of multiwalled carbon nanotubes (MWCNTs) by plant protoplasts. The nanoparticles can form complexes associated with membrane transporters or root exudates, and then nanoparticles are transported into the internal plant structure (Watanabe et al. 2008). The uptake occurs depending on the application way of nanoparticles; if this was on the soil (root entry) or at leaf level (foliar entry), then the exposed tissue of plant organ describes different transport and defense mechanism, and the chemical and physiological responses are specific in each site of contact.

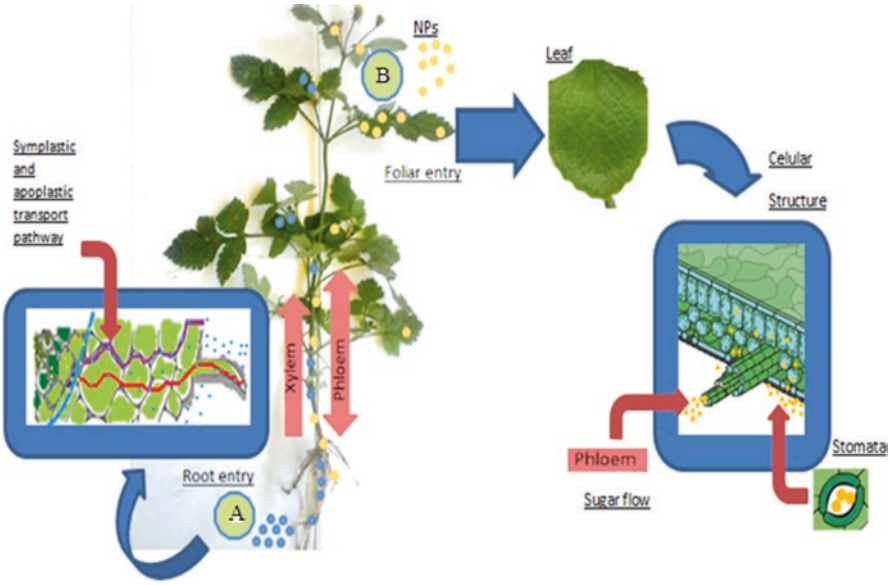

Fig. 4.7 Uptake and transport of nanoparticles in the plant. (A) Root entry: root tips and hairs, rhizodermis, lateral roots. (B) Foliar entry: cuticle, lenticels, hydathodes, wounds, stomata

The diameter of the cell structure pores or transport channels determines the ability to entry for NPs; in roots, for example, there is a size exclusion barrier: (a) cell walls for the apoplastic transport pathway (5–20 nm), (b) the Casparian strip transport (<1 nm), or (c) symplastic transport (3–5 nm) (Wang et al. 2016). However, several reports where nanoparticles dimensions were bigger than 20 nm presented uptake and translocation. There are possible explanations for the entry of larger nanoparticles than the exclusion barriers: the formation of new large pores in the cell wall; the rupture of membranes; and some interactions between cations, proteins, and viruses among others that can cause changes in cellular structure (Wang et al. 2016). Another study is Birbaum et al. (2010); they demonstrated that the uptake did not depend on closed or open stomata, or under dark and light exposure conditions, and an interesting finding; there was no translocation into newly grown leaves of cultivated maize plants, after the foliar particle exposure. These investigation results may indicate that the natural entry barriers of some plants could be more resistant against nanoparticle translocation than mammalian barriers.

Shen et al. (2010) reported evidence of endocytosis-like structure in the plasma membrane in an *Arabidopsis thaliana* leaf cell. Other studies probed that MWCNTs were absorbed by the seeds and root systems of the tomato seedlings (Khodakovskaya et al. 2009). Similar findings were shown by Wild and Jones (2009); they detected MWCNTs on the root surface and in the epidermal and root hair cell walls and root cap of wheat seedlings. Cañas et al. (2008) found no uptake of SWCNTs and functionalized SWCNTs (F-SWCNTs) by roots of cucumber seedlings after 48-h treatment.

The SWCNTs were attached to the outer surface of the main root and the secondary roots. Depending on the concentration, the MWCNTs form black groups associated with the cells (Tan et al. 2009). The nanotubes trigger reaction mechanisms similar to those unleashed by plant pathogens, causing cellular mortality (Hyung et al. 2007). Lin et al. (2009) studied the uptake, accumulation, and translocation of NOM-suspended fullerene C_{70} and MWCNT in rice plants; the finding of C_{70} in the form of black aggregates in the leaves suggests that they followed the route of water and nutrient transmission to through the xylem. In mature plants, NOM-C_{70} was predominantly present in or near the vascular systems and the leaves of the stem, while it was observed that the roots were devoid of C_{70}, which supports the assertion of a robust translocation of the roots to the aerial parts of the plant. In a contrast study, Chen et al. (2010) reported that C_{70} hydrophobic fullerenes blocked the cell wall pores in *Allium cepa* cell suspensions, resulting in an insignificant uptake of NPs by cells.

Some interesting studies in rice plants (*Oryza sativa*) documented the uptake and translocation of carbon nanomaterials, and they observed that fullerene C_{70} was easily taken up by roots and transported to shoots through the vascular system, and it could also be transported from leaves to roots (Lin et al. 2009). The same researchers found a similar result for MWCNTs. Hussain et al. (2013), Liu et al. (2009), and Torney et al. (2007) reported the same findings in MWCNTs and CNPs.

Once the nanoparticles have been uptaken by the roots, they follow the same transport routes of nutrients and water. First, they meet the cell wall, where the pores restrict the access of large particles and aggregates, smaller than 5–20 nm (Ma et al. 2010); once internalized the particles can follow the symplastic route; in this pathway the NPs are translocated to leaves. Xylem is the most important way in the distribution and translocation of nanoparticles (Aslani et al. 2014). Transport is facilitated if they interact with other cellular components such as transport proteins, ion channels, etc. (Anjum et al. 2016). There are reports such as Etxeberria et al. (2012), who proved that there is a mechanism of transport via endocytosis; in this work they also demonstrated that the 40 nm ENPs (nanospheres) go into vacuoles, while 20 nm (nanospheres) ENPs remain in the cytoplasm. Another important pathway also reported is via 20–50 nm plasmodesmata; some author has shown the ability of this route to the transport of ENPs that port endosomes or nano-protein complex to neighboring cells (Larue et al. 2012a, b; Wang et al. 2012a, b). The NPs could be integrated passively through the apoplast of the endodermis before reaching the stele (Judy et al. 2012). Wang et al. (2016) demonstrated the xylem- and phloem-mediated uptake, translocation, and distribution of nCuO. The Casparian strip is the last barrier of translocation, and it works keeping of the free transport of NPs; there are several reports in various plant species (Anjum et al. 2016).

The smart property of cell internalization of ENPs of different sizes and compositions has been observed in different plant species (Lin et al. 2009; Liu et al. 2009; Torney et al. 2007), and it was proposed that some of them could be applied as carriers of DNA or other compounds, for example, SWCNTs and mesoporous silica NPs.

When the root absorbs the NPs, they are transferred and accumulated in the mature leaves, because they are closer to the root, so it is the mature leaves that are usually more exposed than the young ones; thus the time of exposure is higher; a few other papers document the translocation of NPs to grains, fruits, and flowers. Anjum et al. (2016) and Lin et al. (2009) showed that C_{70} fullerene is capable of accumulating in *O. sativa* seeds. Studies with SWCNTs in *N. tobacum* plant cell suspensions found their fate in vacuole as well as cytoplasmic strands (SWCNT-DNA) (Burlaka et al. 2015). A great concern for the accumulation of NPs in edible plants is their transmission to the plant's next generation. Lin et al. (2009) reported that C_{70} was detected in the leaf tissues of second-generation rice plants. About biotransformation of nanoparticles C_{70}, SWCNTs, MWCNTs, Fe_3O_4, and TiO_2 NPs, biotransformation was not observed (Rico et al. 2011).

6 Beneficial and Harmful Effects of CNTs in Plants

For the past decades, a variety of experimental information has been presented about the new areas in which nanotechnology has become a priority in many fields of science like physics, chemistry, pharmaceutical science, material science, medicine, and agriculture (Bhushan 2016; Dasgupta et al. 2017; Feizi et al. 2018). In the field of agriculture, a variety of nanomaterials, mostly metal-based nanomaterials (NMs) and carbon-based nanomaterials (CNMs), have been exploited for their absorption, translocation, accumulation, and effects on growth and development of crop plants (Zhao et al. 2017; Oloumi et al. 2018). Nevertheless, as it is still at a relatively early stage of development, the potential risks remain unclear (Chen et al. 2015).

Within the CNMs are found in various structures such as nanodiamond, fullerene C_{60}, carbon onion, nano-horn, carbon dots, graphene, and carbon nanotubes (Baptista et al. 2015; Kumar et al. 2018). Specifically, in agriculture, the carbon nanotubes (CNTs) are used as a carrier for the fertilizers, insecticides, fungicides, nematicides, and herbicides, with a controlled slow, and sustained release resulting in precise dosage (Benelli et al. 2017; Duhan et al. 2017; Athanassiou et al. 2018). Also, recently, the transition metal NP-grown CNTs were used for a wide range of applications including environmental remediation, particularly in the sanitation of contaminated agricultural soil (Ibrahim et al. 2016; Cecchin et al. 2017; Feizi et al. 2018).

The CNTs stand out having unique properties, including specific structural, electrical, mechanical, and thermal characteristics. There are two main forms of CNTs, single-walled (SWCNTs) with a diameter from 0.4 to 3.0 nm and multiwalled (MWCNTs) where their diameters can reach up to 100 nm (Balasubramanian and Burghard 2005). CNTs, for their tubular structure of crystalline outer diameters, may enhance their further uptake and interaction with the biological system (De La Torre-Roche et al. 2013). Therefore, in this section, we expose the benefits of CNTs on the improvement of physiological and biochemical characteristics in plants. However, the adverse effects on the characteristics already mentioned will be discussed. As various authors have argued, it is essential to assess the associated ben-

efits and risks, since studies suggested that the beneficial or negative impact of nanomaterials on crops is dependent upon their size, shape, concentration, chemical composition, solubility, agglomeration, etc. (Mukherjee et al. 2016; Singh et al. 2017; Tripathi et al. 2016; Tan et al. 2018). In this sense, in the course of the discussion, we will comment on substrate means, size CNTs, concentrations of CNTs, type of plants, exposition time and environmental conditions, and in some cases the methodologies used to demonstrate the effects on plants.

In recent documents such as articles, review, book, book chapters, proceedings paper, letter, etc., evidence has been shown that, in plants, both MWCNTs and SWCNTs affect the physiology and biochemistry in diverse ways. Although positive, negative, and contradictory effects of impacts on plants and soil organisms have been reported, it is striking that in some cases in the conclusions and recommendations of the papers, it is suggested that the CNTs can be a new alternative for increasing production, improving botanical characteristics of plants, etc., with an objective of providing a healthy and adequate diet. Figure 4.8 shows the increase in publications of the last 20 years relational with the effect of CNTs and plants, whether edible or nonedible. The search procedure was conducted in the database "Web of Science," using different combinations of keywords. The keywords "nanoparticles," "carbon nanotubes," "CNTs" ("MWCNTs and SWCNTs"), "plants," and "plants edible" were used as search parameters in the "topic" field. In the search for the words CNTs + plant growth, it yielded a total of 617 documents, while the combination of the words NPs + CNTs + plant was 327 documents. Specifically, with the words CNTs + plant growth, 287 were found documents, which over time has been increasing (Fig. 4.9). Finally, the combination of the words CNTs + plant edible were ten documents. The fact of finding few documents using the word edible plant does not mean that few articles have been published, but that many scientific works use or refer to the scientific name of the crop. For Fig. 4.9 in the search in parameters of a topic, for the year 2019, the pursuit of the data was considered until May of the same year. It suggests that there is a priority for knowing the effect of CNTs on plants.

In the last 5 years, several reviews and scientific articles have been presented that describe the effect of CNTs on edible and inedible plants (Mukherjee et al. 2016; Vithanage et al. 2017; Zuverza-Mena et al. 2017; Verma et al. 2019). About changes in physiological and biochemical processes in plants, in conditions of a laboratory, in the studies of Khalifa (2018), it was shown that agar media of MWCNT (25 μg μl^{-1}; average length = 559.54 nm and width = 56.50 nm) for 6 days enhanced root elongation and the plant growth (*Thlaspi arvense* L.), while the growth rate was reduced at higher concentrations (250 μg μl^{-1}). Also, the CNTs exhibited the ability to bind the genomic DNA at higher concentrations of 100 and 200 μg μl^{-1}. Also, in tomato (*Solanum lycopersicum* L.), it was recently reported that at 10 μg mL^{-1} concentration CNTs were able to penetrate the cell membrane and change the gene expression profile of exposed cells (Lahiani et al. 2019). Even, formerly Lahiani et al. (2015) had argued that all carbon-based ENMs could potentially modify the expression level of genes involved in responses to stimuli.

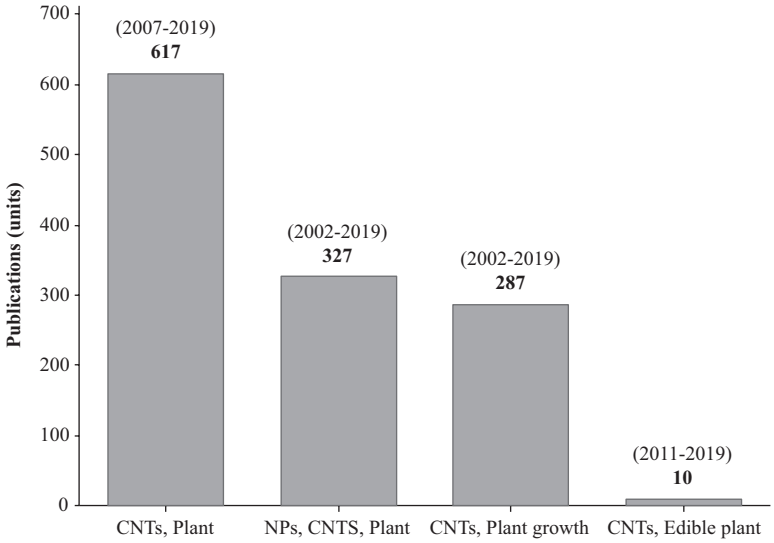

Fig. 4.8 Scientific production for periods. Each of the bars corresponds to the number of publications according to the combinations of words that were searched in the field of topics within the "Web of Science" database

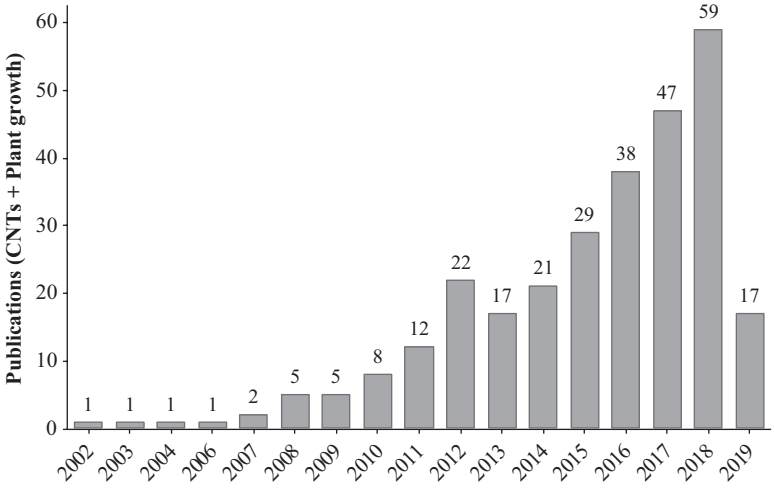

Fig. 4.9 Annual growth of publications relational with carbon nanotubes and plant growth (CNTs + plant growth) in the period 2002–2019 (Web of Science). The search of the data for the year 2019 was considered until May of the same year

In other plants, besides evidencing the presence of CNTs inside the vascular bundles, Joshi et al. (2018a, b) found that MWCNT at 80 and 90 μg mL^{-1} (outer and inner diameter as 10 ± 1 and 4.5 ± 0.5 nm) inside oat (*Avena sativa* L.) enhanced the growth of xylem cells, the chlorophyll content, and the photosynthetic activity. This same author evidenced in wheat (*Triticum aestivum* L.) that because of MWCNT (diameter 13–14 nm), after 135 days, it facilitated the absorption of water and essential minerals such as phosphorus (P) and potassium (K), which increased crop yield by significantly improving grain yield (Joshi et al. 2018a, b). Likewise, Martínez-Ballesta et al. (2016) found that salt-stressed plants (100 mM NaCl) increased water uptake due to the positive effect of MWCNTs at 0.10 mg L^{-1} (average size of 0.1–0.5 μm length). Also, there were induced changes in the lipid composition, rigidity, and permeability of the root plasma membranes relative in *Brassica oleracea* L. var. Italica.

In a hydroponic system, the absorption of MWCNT by *S. lycopersicum* fruits significantly affected the total fruit metabolome (McGehee et al. 2017). By contrast, Khodakovskaya et al. (2009) in microcosms experiment with *S. lycopersicum* showed that CNTs (10–40 μg mL^{-1}) positively affect seed germination and growth of tomato seedling. Mondal et al. (2011) revealed that the MWCNTs actively enhance seed germination by penetrating the seed coat, as reported in germinating tomato seeds, and increasing the growth of *Brassica juncea* L. Likewise, in different substrate media and environmental conditions, also it has been reported that CNTs have the capacity to increase stems, leaves, and root growth, as well as seedling development of *S. lycopersicum* (Ratnikova et al. 2015). However, contrary to these authors, others have demonstrated adverse effects (Haghighi and da Silva 2014), and null effects have been reported (De La Torre-Roche et al. 2013).

On the other hand, Kumar et al. (2018) showed in *Allium cepa* L. that CNTs in ZnO/MWCNTs nanocomposites at 15 μg mL^{-1} displayed the best seedling growth with maximum number of cells in telophase and the growth was proportional to the increase in the concentration of ZnO/MWCNTs with a negative impact on plant growth in contrast to the use of MWCNTs. To evaluate uptake of CNTs of agriculture crops, Das et al. (2018) used both pristine (p)-MWCNT and carboxyl-functionalized (c)-MWCNT (20 mg L^{-1}). The results showed that both the MWCNT types were found to be present in the leaf, stem, and root tissues of the treated *Lactuca sativa* L. plants confirming their uptake and translocation in the plant. Other aspects and examples related to absorption and uptake can be seen in one of the previous sections.

CNT has been used by several years by phytoremediation. In the studies realized by Gong et al. (2019), it was shown that in *Boehmeria nivea* L., MWCNTs at 500 mg kg^{-1} promoted the accumulation and translocation of Cd and alleviated Cd-induced toxicity by stimulating plant growth, reducing oxidative stress, activating antioxidant enzyme activities, and increasing specific antioxidant content. In other experiments of phytoremediation, Oloumi et al. (2018) found that in *Brassica napus* L., total chlorophyll (Chl) content increased with MWCNTs (10 nm) at 10 and 50 mg L^{-1} exposure under cadmium or lead stress and at 10 mg L^{-1} mitigated the deleterious effects of Cd ions on total chlorophyll content of *Helianthus annuus*

L. and *Cannabis sativa* L. Contrary to previous studies, Shen et al. (2018) reported that in hydroponic conditions contaminated (lead) cultivated with *Cucumis sativus* L., the increase of MWCNTs from 0 to 1000 mg L^{-1} effects on pyrene bioaccumulation in cucumber seedlings. The results suggest that enhanced transport was due to analyte movement with internalized MWCNTs driven by transpiration within the plant. Also, for the authors, these findings are important for evaluating the potential risk of MWCNTs in food safety, especially for crops grown in organic pollutant contaminated areas.

Other experiments with negative effect have been demonstrated by Chen et al. (2015), with an in vivo sampling technique. Its study revealed the impact of MWCNTs (inside and outside diameters, 35 nm and 10 nm) on the accumulation/depuration behaviors of contaminants in a crop of mustard (*Brassica juncea* L.). The results show enhancement of contaminant accumulation in living plants, but some large black spots observed under light microscopy indicated that the MWCNTs aggregated within the roots, which might cause negative effects, inhibiting nutrient transport and affecting plant growth. Although CNTs can be considered plant growth promoters, the author suggests that this occurred only at a low concentration, because higher concentrations in exposure time can be phytotoxic (Vithanage et al. 2017). By contrast, Hao et al. (2016) found that MWCNTs and Fe-CNTs significantly decreased the biomass production rate at 30 and 50 mg L^{-1} in crop rice, whereas Wang et al. (2012a, b) reported that 7 days of exposure to the o-MWCNTs medium (6–13 nm) at 10–160 μg mL^{-1}, faster root growth and higher vegetative biomass were observed, but seed germination and stem length did not show any difference as compared with controls. For what has been discussed, it is observed that there is a diversity of effects that cause the CNTs (+ vs. −); even within the same organs of a plant, there are different answers. For example, Fan et al. (2018) revealed that 50 mg L^{-1} MWCNT could have a positive effect in lateral root number and photosynthesis, but a toxic effect on the root growth of *Arabidopsis thaliana* plants. Other examples of MWCNTs effects in plants can be in Table 4.4.

Table 4.4 Positive and negative effects of MWCNTs on physiological and biochemical characteristics in plants

Concentrations	Size (nm)	Species of plant	Substrate medium	Description of the effects	References
Positive effects					
40 μg mL^{-1}	NR	*Lycopersicon esculentum Mill.*, var. Arka Vikas	MS medium	Increased seed germination and plant growth	Morla et al. (2011)
0–60 mg L^{-1}	NR	*Zea mays* L.	BA medium	Enhance the germinative growth of maize seedlings at low concentrations but depress it at higher concentrations	Tiwari et al. (2013)

(continued)

Table 4.4 (continued)

Concentrations	Size (nm)	Species of plant	Substrate medium	Description of the effects	References
0–100 μg mL^{-1}	8 nm	*Oryza sativa* L.	Standard agar and MS medium	Increased stem and root length of the paddy seedlings	Jiang et al. (2014)
0.0, 0.05, and 0.1 mg L^{-1}	100–170 nm	*Phoenix dactylifera* L.	Peat moss and perlite	Facilitate the absorption of nutrients and their transportation into the plant tissues	Taha et al. (2016)
5–50 mg L^{-1}	20–40 nm	*Oryza sativa* L.	Hoagland medium	Promoted rice root growth through the regulation of expression of the root growth-related genes	Zhang et al. (2017)
Negative effects					
1000 mg L^{-1} and 2000 mg L^{-1}	NR	*Spinacia oleracea* L, *Lactuca sativa* L, and *Cucumis sativus* L.	Hydroponic medium	Reduced the root and shoot lengths	Begum et al. (2014)
0, 5, and 10 μg mL^{-1}	NR	*Allium cepa* L.	High-performance liquid chromatography (HPLC) analysis of DNA	Altered cellular morphology, destroyed membrane integrity, and disrupted mitochondrial function in root cells	Ghosh et al. (2015)
10, 100, and 1000 mg kg^{-1}	20 nm	*Trifolium pratense* L.	Agricultural soil	Decreased number of flowers	Moll et al. (2016)
150 mg L^{-1}	Nitrogen co-doped MCNs: MCN1 (150) MCN2 (80 nm)	*Oryza sativa* L.	Hydroponic medium	The decrease in root length and shoot length	Hao et al. (2019)

Note: *NR* not reported, *MS* Murashige and Skoog medium, *BA* bacteriological agar

In the context naturally, the activation of MWCNTs during forest fires impacts the development of the forest plants, as suggested by Lara-Romero et al. (2017). In laboratory conditions it was found that MWCNTs synthetic (30 μg mL^{-1}; diameter, 6–13 nm) exhibited increased germination rates of 62.5% and 40% compared to untreated seeds in *Lupinus elegans* Kunth and *Eysenhardtia polystachya* (Ortega) sarg., respectively. However, when they collected burnt wood of *Pinus oocarpa* Schiede ex Schltdl and it was examined by thermogravimetric analysis (TGA) and HR-TEM images and fast Fourier transforms (FFTs), the samples indicated the presence of CNTs. The results were strongly suggesting a possible impact on natural plants of the resinous forest ecosystems (*P. oocarpa*) through their effects on seed germination and plant growth promotion.

Regarding the SWCNTs it has been shown that increased growth of tobacco cells (78% increase compared to control) as well can activate seed germination of selected crops and enhance growth of different organs of *Zea mays* L., *Solanum lycopersicum* L., *Oryza sativa L.*, and *Glycine max* (Lahiani et al. 2015). Analogous results were achieved by Tripathi et al. (2016) who demonstrate that SWCNTs post 7 days stimulate the growth of roots and shoots in *Cicer arietinum* L. Contrary results were reported by Hao et al. (2016), indicating that the SWCNTs significantly decreased N assimilation and negative effect in plant hormones concentration to rice (*O. sativa* L.), whereas in inedible plants, Hatami et al. (2017) found that SWCNTs at low concentrations induced tolerance in seedlings against low to moderate level of drought by enhancing water uptake and activating plant defense system. Other examples of SWCNTs effects in plants can be seen in Table 4.5. There are several methodologies, media of substrates, and concentrations to evaluate the effect of CNTs on edible and inedible plants, all of the above supported by analysis of chemical digestion and Raman analysis, among others. However, it is necessary to continue contributing with improvements in the methodologies and specific edible crops mostly consumed by man and thus be able to know both the possible toxic effect on the plant and the food chain.

Table 4.5 Positive and negative effects of SWCNTs on physiological and biochemical characteristics in plants

Concentrations	Size (nm)	Species of plant	Substrate medium	Description of the effects	References
Positive effects					
20 mg L^{-1}	1–2 nm	*Zea mays* L	MS medium	Accelerate maize seminal root growth	Yan et al. (2013)
4 μg mL^{-1}	1–2 nm	*Rubus adenotrichos* L.	In vitro (glass flasks)	Promoted the growth of the in vitro plants under this assay	Flores et al. (2014)
50 g mL^{-1}	1–3 nm	*Hyoscyamus niger* L.	Petri dishes	Enhancing water uptake and activating plant defense system	Hatami et al. (2017)

(continued)

Table 4.5 (continued)

Concentrations	Size (nm)	Species of plant	Substrate medium	Description of the effects	References
Negative effects					
5–250 μg mL^{-1}	1–2 nm	*Arabidopsis thaliana* L.	Cell culture	Caused adverse cellular responses including cell aggregation, chromatin condensation, and plasma membrane deposition	Shen et al. (2010)
15, 25, 50, 100 μg mL^{-1}	NR	*Arabidopsis thaliana* L.	Petri dishes	Exhibited obvious toxic effects to the protoplasts such as the increasing generation of ROS, inducing changes of protoplast morphology, changing green leaves into yellow	Yuan et al. (2011)
400–800 μg mL^{-1}	1–3 nm	*Hyoscyamus niger* L.	Petri dishes	Inhibited seed germination and seedling performance, increased cellular injury indices, and changed antioxidant enzyme activities	Hatami et al. (2017)
0.1 and 1.0% of SWCNTs	10–20 nm	*Ferocactus latispinus* Britton and Rose, *Melocactus matanzanus* Leon, and *Parodia ayopayana* Cárdenas	The black soil, sand, peat, and finely ground (ca. 1 mm) red volcanic rock	Pristine arc-discharge SWCNTs exhibit the strongest phytotoxicity at 40 weeks	Basiuk et al. (2018)

Note: *NR* not reported, *MS* Murashige and Skoog medium

7 Effects of CNTs on Soils and Their Organisms

For two decades, the advance of nanotechnology has progressed by leaps and bounds. Nanotechnological innovations have been used to cure diseases, in biomedical instrumentation, nanosensors, biomarkers, visualization devices, agricultural technology, environmental protection, etc. About the agricultural area, nanotechnology has solved many of the bottlenecks compared to conventional systems, in terms of improving the production, physiological and biochemical characteristics of plants, and the control of diseases, pests, and weeds (Verma et al. 2018).

Even authors such as Jakubus et al. (2017) point to the group of carbon nanotubes (CNTs) as promising in agricultural and industrial applications. Within this group, three kinds of CNTs exist: single-walled CNTs (SWCNTs), double-walled CNTs (DWCNTs) with two concentric tubes, and multiwalled CNTs (MWCNTs) with more than two concentric tubes. CNTs diameter varies from a few nanometers for SWCNTs to several tens of nanometers for MWCNTs. Their length is usually of a few micrometers. CNTs have remarkable optical, electrical, thermal, mechanical, and chemical properties that make them unique among nanomaterials at the nanolevel and of great importance for their handling and application (Liné et al. 2017). Nevertheless, despite the great benefits of CNTs, adverse effects on the environment have gradually emerged (Chen et al. 2017a, b).

It is well known that the soil is the basis of multiple ecosystem services, such as human nutrition, climate regulation, and the nutrient cycle, among others (Cai et al. 2015; Pachapur et al. 2016). Soil microbial communities play an important role in nutrient cycling, environmental pollutant removal, and maintaining the stability of basic soil characteristics, which are sensitive indicators of soil responses to environmental stressors, such as heavy metals, pesticides, and nanomaterials (Chen et al. 2017a, b). In this line, currently, several authors have discussed the effect of CNTs on microorganisms (Simonin and Richaume 2015; Chen et al. 2019; Maksimova 2019), in the association plant-microorganisms (Achari and Kowshik 2018; Hao et al. 2018). Also, authors such as Liné et al. (2017) in your review have debated and have shown evidence of the adverse effects of CNTs on the plant, soil, and organisms. There is a greater concern since it is estimated that by the year 2030, almost 40 tons/year can be transferred to the soil (Das et al. 2018). However, Qian et al. (2018) argue that few studies confirmed the effect of CNTs on the physicochemical properties of the soil and its organisms, specifically on bacterial communities or the details of the relationship between the diversity of soil microbial communities. Even Liné et al. (2017) in your review mentioned that of 71 studies on terrestrial ecosystems examined, the studies on soil microorganisms and macroorganisms covered to 14% and 17% of the total studies and the less studied is the behavior of CNTs in soil (in laboratory soil column) with only 4% of the mentioned articles. Therefore, we searched for the "Web Science" database. When we focus on the topics section, we find several documents that relate the word "CNTs" and "soil" (856 documents). When the search was conducted in the title field, only 62 documents were found, while in the combinations of "CNTs," "soil," and "remediation," the search showed 60 documents, but when we use other words and the combinations between them, such as "CNTs" and "soil microorganisms," the database shows less of publications (11 documents). We see that due to concerns about the pollution of the environment and the need to understand complex systems, research continues, and the effect of nanomaterials (NMs) is not understood at all.

Depending on their length, diameter, functionalization, and environmental conditions, CNTs may have different behavior in natural conditions (Jackson et al. 2013). However, the detection and quantitative analysis of CNTs in biological samples are very complex because it is difficult to detect a specific form of carbon in a carbon-based matrix (Bourdiol et al. 2013). Due to the above, quantitative measurements of

CNTs in key environmental matrices (water, soil, sediment, and biological tissues) are needed to address concerns about their potential environmental and human health risks and to inform application development (Petersen et al. 2016). Although in this section, our intention is not to demonstrate the techniques involved in the quantification and analysis of CNTs, we will mention some examples of the behavior of CNTs in the soil. So that, among the studies that relate the behavior of the CNTs and the soil matrix is that of Bennett et al. (2013) who have reported that surfactants and natural organic matter (NOM) stabilize CNTs in the aqueous phase, thereby enhancing material mobility in soil systems. The soil properties such as pH, clay, and organic carbon content, texture, and mineralogy could affect CNM mobility in the environment (Avanasi et al. 2014). Lu et al. (2014) found that positively charged MWCNTs were entirely retained in soils, while negatively charged CNTs broke through the soil column and were found in the outlet. Also, it demonstrated that soil texture, rather than organic matter, controlled MWCNT mobility.

On the other hand, Shan et al. (2015) showed that SWCNTs (2000 mg kg^{-1} dry soil) reduced mineralization, while MWCNTs at 0.2 mg kg stimulated mineralization compared with the control soil. The inhibitory effects of SWCNTs on the mineralization were attributed to the inhibited soil microbial activities, and the stimulatory effects of MWCNTs on the mineralization were attributed to the selective stimulation of specific catechol degraders by MWCNTs at 0.2 mg kg^{-1}. In general, the CNMs reaction or effect in the soil environment is difficult to know. In addition, the study in the soil becomes more complicated since several highly correlated soil factors must be taken into accounts, such as their mineral and organic composition and the structural heterogeneity of the soil to understand the transport and fate of the nanoparticles, and CNM properties and the identity/susceptibility of potential receptors are complex.

Naturally, soil organisms can eliminate, attenuate, degrade, transform, or break down (through metabolic or enzymatic action) the undesirable substances to inorganic components. For example, polycyclic aromatic hydrocarbons (PAHs) can be dissipated through biodegradation and bioremediation processes (Fernández-Luqueño et al. 2017). Recently, they have used the capacity of organisms to be incorporated into nanotechnology. This technique is known as nanoremediation (Cecchin et al. 2017). Indeed, several studies have reported that due to their unique physicochemical characteristics (e.g., large surface area, high microporosity, and superb sorption capacities), the effective role of CNTs increased occurrence in the environment and potential value in remedying contaminated soil and sediments (Abbasian et al. 2016).

Nevertheless, widely varying impacts both positive and negative of CNTs have been reported, possibly by their application as *adsorbents* or membranes (Song et al. 2018) (Fig. 4.10). For example, Qian et al. (2018) revealed that the SWCNTs at concentrations of 3 and 10 μg g^{-1} change the composition of soil microorganism communities, promote soil organic degradation, and improve soil fertility in a short time. Likewise, Song et al. (2019) reported that the incorporation of 0.5% MWCNTs into the contaminated sediment with phenanthrene significantly enhanced microbial activity compared with the blank control. To evaluate the CNTs in contaminated soil

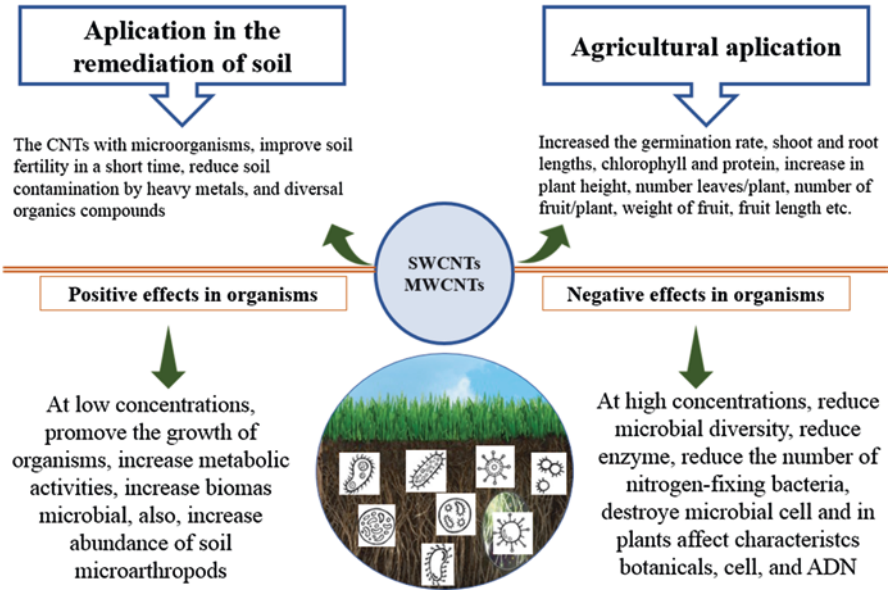

Fig. 4.10 Effects of CNTs on soils and their organisms

with oil alone for 30 days, Abbasian et al. (2016) found that a combination of crude oil and low concentrations of carbon nanotubes can increase the diversity of the total microbial population. Likewise, Ge et al. (2018) showed that only concentrations lower of MWCNTs could affect the vegetative stage of plants and microbial communities, which was manifested in an increase in the number of bacteria. By contrast, from long ago, Chung et al. (2011) showed that high concentrations of MWCNTs (5000 μg MWCNT g^{-1} soil) decreased the microbial activity and biomass in soils. Likewise, Kerfahi et al. (2015) compare the effect of both raw and acid-treated or functionalized MWCNTs on soil bacterial communities. The results show that soil bacterial community composition was affected only by functionalized MWCNTs at high concentrations, while raw MWCNTs did not affect the composition of the soil microbial community.

Despite the benefits of nanotechnology, it is necessary to consider the CNTs interaction in the environment. Currently, utilizing other types of NMs, Yang et al. (2016) proposed an empiric model based on the use of nematode *Caenorhabditis elegans* to determine the effect of the Fe^0 ENPs on soil health. The authors concluded that *C. elegans* biomarker-based risk model affords new insights into the links between the widespread use of Fe^0 ENPs and ecological implications of metal-based NPs for in situ remediations. Subsequently, to evaluate the toxicity of Ag NPs on organisms, Yang et al. (2017) confirmed the effectiveness of *C. elegans* as a proxy for estimating soil risk metrics can help develop methods of management for mitigating the metal NP-induced toxicity on terrestrial ecosystems.

In the association edible plant-organisms, Yuan et al. (2017) showed that under conditions of substrate artificial soil (perlite and vermiculite at 1:1 volume ratio), the effects of four carbon-based materials (activated carbon (AC), SWCNTs, MWCNTs, and graphene oxide (GO) on the rhizobium-legume symbiosis system consisting of *Lotus japonicus* and *Mesorhizobium loti* MAFF303099) were studied. The results showed that at 100 μg mL^{-1} MWCNTs increased by 39% at 14 days the number nodules, and the biological nitrogen fixation of the nodules was promoted by more than 10% under 100 μg mL^{-1}. In other studies, Bai et al. (2017) investigated the influence of graphene (G), GO, and CNTs on microarthropod soil communities under turfgrass growth conditions. The results show that the application of carbon nanomaterials resulted in increased abundance of all soil microarthropods, especially in the GO and CNTs treatments. GO also significantly increased the abundances of multiple trophic functional groups, including predators, detritivores, herbivores, and fungivores.

By contrast, contradictory effect caused by CNTs exists; in some cases MWCNTs both promote and inhibit the growth of microorganisms. This effect depends on the type of microorganisms, the external environment, and the concentration and structure of MWCNTs. Compared with other factors, surface sorption capacity is influenced by different sizes and functionalization of MWCNTs (Chen et al. 2019). For example, Hao et al. (2018) showed that after 30 days of exposure, all the three CNMs negatively affected the shoot height and root length of rice, significantly decreased root cortical cells diameter, and resulted in shrinkage and deformation of cells, regardless of exposure doses (50 or 500 mg kg^{-1}). Also, it revealed that the presence of CNMs significantly altered the composition of the bacterial community. In other studies, Moll et al. (2016) show that MWCNTs decreased the total number of flowers of red clover when applied to the soil at the concentration of 3 mg kg^{-1} and increased biological nitrogen fixation by 8% at the concentration 3000 mg kg^{-1}, but had no impact on plant biomass or root colonization by arbuscular mycorrhizal fungi (AMF).

Therefore, it is observed that in nanotechnology, although it is considered advanced technology, many questions about the advantages and disadvantages that can be generated by the use of nanomaterials have emerged. It is there is more than what is observed on the surface of the soil; there is a diversity of soil biota that can be damaged without taking into account the fundamental role in soil fertility, nitrogen release, climate regulation, etc.

8 Conclusion

Carbon nanotubes (CNTs) have an effect on the plant growth, but there is not a consensus regarding if this is positive, negative, or null. However, this evidence does not match between themselves because there are several biotic or abiotic factors that affect the crop performance, coupled with the intrinsic effects of CNTs. So, some variables such as the number of walls, concentration, size, exposition time,

plant phenological state at the addition of CNTs, and species of the crop, among others, will affect the crop development and the synthesis of plant growth regulators. Metabolomics or other scientific areas such as OMICs could be very important to characterize not only the synthesis of plant growth regulators but also the metabolic pathways involved in the synthesis of a specific compound. Therefore, the characterization of plant growth regulators by crops amended with CNTs could promote the molecular farming of high-value compounds synthesized by plants. It has to be remembered that environmental concerns regarding CNTs recently taken center stage in policy and scientific discussions around the globe due to their frequently observed impact on the human and environmental health. Unfortunately, environmental concerns have always met with difficulties to become a priority worldwide because these have remained as a secondary priority for governmental or social organizations.

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Chapter 5
Nanobiosensors for Bioclinical Applications: Pros and Cons

Raouia Attaallah, Amina Antonacci, Fabiana Arduini, Aziz Amine, and Viviana Scognamiglio

Contents

1 Introduction

Nanotechnology has a significant influence on our economy and society by providing significant advances in diverse areas, including manufacturing, nanoelectronics, medicine and health, energy, biotechnology, information technology

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and security (Reese and Reese 2013). For the biomedical sector in particular, there is a universal promise that nanotechnology will provide the next industrial revolution. For example, nanomaterials are ideally suited for use as drug administration systems, enabling advances in new theranostics with highly sensitive detection capabilities (Maduraiveeran et al. 2018). Thanks to unique nanomaterial properties and their nanosize, new diagnostic devices (intelligent biosensors) able in detecting minute concentration of a desired analyte are emerging. To this aim, nanomaterials may be exploited as (i) transducer materials, playing a significant role in the development of biosensors (Lan et al. 2017), (ii) bioaffinity platforms for the immobilization of biomolecules (DNA, enzymes, antigens or antibodies) or (iii) electrode modifiers (Bravo et al. 2017; Yong Zhang and Wei 2016). The intelligent application of nanomaterials is intended to improve the performance of miniaturized devices in terms of sensitivity, selectivity and stability (Choi et al. 2007; Kurbanoglu and Ozkan 2016; Maduraiveeran et al. 2018; Li et al. 2015). Indeed, nanomaterials offer excellent electrical conductivity to amplify desired signals and good biocompatibility with biological molecules (Fernández-baldo et al. 2009; Zhang and Wei 2016). The most important clinical applications involving currently available nanomaterials encompass DNA detection assays, discovery of biomarkers, cancer diagnoses and recognition of infectious microorganisms (Bravo et al. 2017; Maduraiveeran et al. 2018). Thanks to this wide application range, nano(bio)sensors will have a huge impact on conventional medical practices by enabling early ultrasensitive diagnosis as well as long-term monitoring of clinical biomarkers by means of point-of-care diagnostics and home healthcare instrumentation. Herein, we report an overview of the nano(bio)sensors developed in the last few years, thanks to the exploitation of nanomaterials as signal amplifiers, bioreceptor labels and support modifiers, as well as to design nanoelectronics, microfluidics, lab-on-a-chip and point-of-care devices.

2 Nanotechnology as a Revolution in Biosensor Design

The International Organization for Standardization defines the term “nanomaterial” as a material with an external dimension or internal structure or at the nanoscale (1–100 nm). Nanostructured materials have gain momentum in the last decades due to excellent mechanical, electrical and optical characteristics conferred by their nanoscale dimensions as well as the perfect combination of volume and surface properties with overall behaviour (Aricò et al. 2005). Their use as electrode materials has a dual function: they act as electrocatalysts by reducing the energy barrier for electrochemical reactions and promote the transfer of electronic charge that takes place on surfaces (Ren and Tilley 2007). General methods are required to enforce architectural order in multidimensional nanomaterials composed of nano-objects of different shapes and sizes, such as points, spherical particles, bars, wires, horns, sheets and other unspecified geometric architectures.

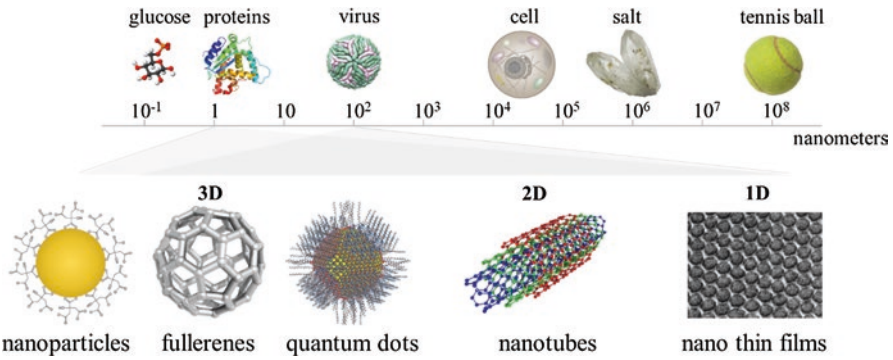
Nanomaterials are generally divided into three classifications:

- One-dimension nanomaterial (1D) such as thin film or monolayer with size less than 100 nm.
- Two-dimension nanomaterial (2D) such as carbon nanotubes.
- Three-dimension nanomaterial (3D) such as quantum dots, fullerenes and metal nanoparticles.

Scheme 5.1 highlights the dimensional size of various nanomaterials.

A rich assortment of nanomaterials with well-controlled physicochemical characteristics, surface charge, shape and dimensions are fashioned by significant advances in synthetic methodologies for sensor applications. Indeed, nanomaterials have unique optical and electrical characteristics that make their incorporation into sensor configurations particularly attractive by allowing numerous benefits (Boverhof et al. 2015). Thanks to the large reactive surface and small particle size, sensors based on nanomaterials show important benefits by a physical, chemical and biological point of view (Farka et al. 2017). A higher surface-to-volume ratio allows for better catalysis and detection response and also improved optical, magnetic and electrical features, well-fitting with biomedical applications (Piscitelli et al. 2016).

In medical diagnosis and clinical analysis sectors, the usage of nanosensors to determine specific anatomical sites or scrupulous cell types in the human body is potentially expanding. Nanosensors provide high sensitivity and ease of miniaturization, which can help to project a new model for clinical and field-deployable analytical instruments (Kneipp 2017). As above-mentioned, the main clinical applications involving nanomaterials currently available include DNA testing, biomarker discovery, cancer diagnosis and recognition of infectious microorganisms. A comprehensive variety of nanomaterials can be exploited for theranostic purposes (Table 5.1); they have strong binding affinities for various biomolecules and drug targets and have the ability to diagnose and treat serious diseases such as cancer and HIV/AIDS. Carbon-based nanomaterials (e.g. carbon nanotubes, fullerenes,



Scheme 5.1 Nanomaterials and their nanodimensions

Table 5.1 Nanomaterial-based electrochemical sensors for biomedical applications

Nanomaterial	Analyte	Linear range/detection limit	References
MWNT-GO	Glucose	28 μM	Palanisamy et al. (2014)
AgNPs-MoS_2	Dopamine	1 μM to 500 μM	Sookhakian et al. (2018)
AuNPs/rGO-Pt	Dopamine	16.57 nM	Chen et al. (2019)
NH2-Fe3O4 NPs-ErGO/GCE	Rutin	0.1–8.0 μM	He et al. (2019)
pAuNPs	Avian influenza virus	1 pM	Lee et al. (2019)
AuNPs	Human serum albumin	0.01 μg/mL	Liu et al. (2019)
MWNT/Ag-TiO2	DNA	1×10^{-11}–1×10^{-6} M/3.12 pM	Smith et al. (2019)
ZnO-Pt/CNTs	Epinephrine	0.1 μM and 0.5 μM	Samadzadeh et al. (2019)
rGO-MWNT	Cholesterol	100 fM	Basu et al. (2019)
GCE/GQDs/AuNPs	Norepinephrine	0.5 and 7.5 μM/0.15 μM	Tapia and Segura (2019)
ZnO-MWNT	Paracetamol	0.79 μM	Kumar et al. (2019)
MWNT-GONPs	microRNA-21	0.034 fM	Lu et al. (2019)
Gold nanoparticle	DNA	8.0×10^{-17}–1.6×10^{-12} M/28 aM	Jensen et al. (2011)
Gold nanoparticle	microRNA-21	200 pM to 388 nM/100 pM	Mandli et al. (2017)
Silver nanoparticle	H_2O_2	5.0×10^{-5}–6.5×10^{-3} M/2.7×10^{-5}	Hu et al. (2008)
Ag nanoparticles-MWNT-COOH	DNA	9×10^{-12}–9 nM/3.2×10^{-12} M	Zhang et al. (2009b)
Silver nanoparticles	Carcinoembryonic AG	5 pg mL^{-1}–5 ng mL^{-1}/3.5 $pgmL^{-1}$	Lai et al. (2012)
Silver-DNA hybrid nanoparticles	Glucose	0.1–1.7 mM/4 mM	Wu et al. (2006)
TiO_2 nanotube-Ni composite	Glucose	0.01–15.2 mM/2 mM	Wang et al. (2010)

graphene) can be considered as the most exploited in electroanalytical and electrocatalytic detection applications. Noble metal nanoparticles are also broadly employed as nanomaterials for biosensing approaches, as well as in other biomedical applications (Chen and Chatterjee 2013).

In this sense, there is a wide literature describing the design of nanosensors for the clinical sector; however, many drawbacks still require to be attempted. For example, the increasing need for analytical instruments requiring smaller sample

volumes, reduced energy consumption and improved performance has been a potent incentive behind the rapid growth of nanomaterial research. Also, the conception of nanomaterials able to interact with organic/biological compounds and specific polymers is currently facing significant challenges. Conversely, the connections between nanomaterials and entity support matrices and critical structural parameters can also affect the catalytic and biosensing features of nanomaterials (Rong et al. 2017). The following sections deal with the wide plethora of nanomaterials available for sensor development and main examples of diagnostic tools for applications in the biomedical sector.

2.1 Carbon-Based Nanomaterials

2.1.1 Carbon Nanotubes

Carbon-based nanomaterials, including single-walled carbon nanotubes (SWCNT), multi-walled carbon nanotubes (MWCNT), single-walled carbon nanohorns (SWCNHs), buckypaper and graphene, among others, offer many important benefits because of their excellent features, as excellent surface-to-volume ratio, electrical conductivity, chemical stability as well as good biocompatibility and strong mechanical strength (Chen and Chatterjee 2013; Erol et al. 2017; Kim et al. 2017; Teradal and Jelinek 2017). Carbon nanomaterial-based biosensors can furnish better sensitivity and stability than their unmodified homologues. Carbon nanohorns and carbon nanofibres are also nanomaterials explored as new and biocompatible matrices for the manufacture of biosensor devices (Chen and Chatterjee 2013).

Among carbon nanomaterials, CNTs have a distinctive combination of mechanical, electrical and optical properties with the capacity to replenish them with various compounds, including drugs (Simon and Flahaut 2019). Carbon nanotubes are an allotropic form of carbon recognized in 1991 by Iijima and since then extensively studied and used for wide-ranging applications such as the reinforcement of materials, electrode materials and/or components for nanoelectronics (biosensors) or even biomedical and pharmaceutical supports (Simon and Flahaut 2019). Carbon nanotubes can be classified as single-walled nanotubes (SWNTs), double-walled nanotubes (DWNTs) and multi-walled nanotubes (MWNTs) according to the quantity of graphite layers.

SWNTs, composed of single graphene sheets that are seamlessly wrapped in cylindrical tubes with a diameter between 0.4 and 2.5 nm, show outstanding physical and chemical features that allow a huge range of biomedical applications (Chen and Chatterjee 2013). Their high electrical conductivity, joint with their small size, allows them to become suitable individual nanoelectrodes; many studies have shown SWCNT capability to effectively promote electron transfer reactions. Yu et al. (2006) reported the design of electrochemical immunosensors using SWCNT

forest platforms with multi-labelled secondary antibody and nanotube bioconjugates for detection of cancer biomarkers in serum and tissue lysates with high sensitivity. An amplified sensitivity was achieved by applying bioconjugates containing horseradish peroxidase (HRP) markers and secondary antibodies (Ab(2)) bound to carbon nanotubes with a high HRP/Ab(2) ratio. This approach yielded a detection limit of 4 pg mL^{-1} for prostate-specific antigen (PSA) in 10 μL of undiluted calf serum. This immunosensor proved its promising potential in clinical screening of cancer biomarkers.

MWCNTs are also employed in biological systems due to their ability to easily penetrate through the cell membrane, their sustained capacity and their distribution in cells. MWCNTs have a diameter up to 20 nm (Kechagioglou et al. 2019), and they can be used for diverse applications, including protein, gene and drug delivery and diagnostics. They have been used to build an electrochemical sensor to detect DNA molecules in the calf thymus (Chen and Chatterjee 2013). Wang et al. (2003) developed a biosensor constituted of a gold electrode modified by MWCNT for glucose oxidase immobilization, which indicates high sensitivity and stability in glucose detection and demonstrates that MWNT is a good platform for enzyme immobilization in biosensor construction.

Ni nanoparticles were immobilized on functionalized MWCNT (Ni-MWCNT) by Baskaya et al. in the design of a non-enzymatic glucose sensor (Başkaya et al. 2017). The Ni-MWCNT-based sensor provided a good response within a linear range of 0.05–12.0 mM and with a low detection limit of 0.021 μM. The novel surface structure, the defined interfaces between Ni and MWCNT and the described large surface area improved electrochemical detection performance, which also showed high stability.

2.1.2 Graphene

Inspired by the achievement of CNTs, graphene has been broadly described in the last few years as new nano-drug carrier for loading a variety of therapies, including anti-cancer drugs, poorly soluble drugs, antibiotics, antibodies, peptides, DNA, RNA and genes (Liu et al. 2013). Graphene is a two-dimensional (2D) carbon lattice with a hexagonal lattice resembling a honeycomb structure that shows high sensitivity, great selectivity, good stability, low overvoltage, large potential window, negligible capacitive current and excellent electrocatalytic activity (Yu et al. 2017).

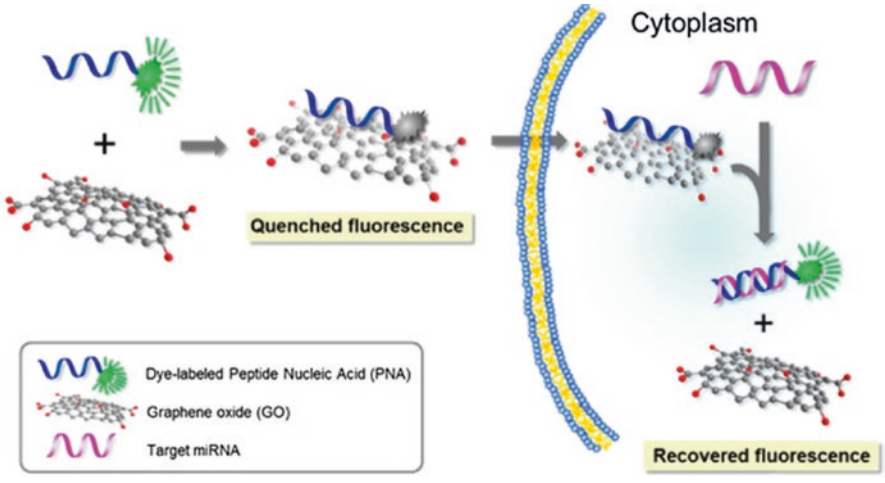
These crucial features make graphene a great candidate in the design of both electrochemical and optical (bio)sensors, being able:

(i) To adapt the electrochemical assets of a printed electrode and to work as label/loading agent for biomolecules and nanomaterials, thanks to its high surface area and easy functionalization, in the case of electrochemical transduction.
(ii) To provide fluorescence quenching at any wavelength by means of energy transfer in the case of optical transduction.

Pumera and co-workers described the astonishing features of different graphene-based materials (Pumera 2010) in the design of (bio)sensors to detect the most important bioclinical markers such as dopamine, glucose, hydrogen peroxide and NADH (Pumera et al. 2010). Bahadır and Sezgintürk (2015) reviewed the realization of different kinds of electrochemical biosensor-based graphene in joint combination with enzymes, antibodies and DNA for the detection of several clinical biomarkers, demonstrating the high potential of graphene for the effective and robust immobilization of the bioreceptors.

Govindhan et al. (2015) designed an electrochemical β-nicotinamide adenine dinucleotide (NADH) sensor using unscrewed gold nanoparticle/reduced graphene oxide (AuNPs/rGO) without using redox mediators and enzymes. The AuNPs/rGO sensor showed superior electrocatalytic activity towards NADH oxidation in neutral solution by providing a suitable atmosphere for electron transfer owing to the augmented electrical conductivity. This sensor showed high sensitivity (0.916 μA/μM cm^2) and wide linear range (50 nM–500 μM) with a low detection limit of 1.13 nM (S/N = 3).

Ryoo and co-workers (2013) exploited graphene oxide to realize an optical biosensor for the determination of microRNAs exploiting peptide nucleic acids (PNA), highlighting the high loading capacity of graphene for bioreceptor immobilization. In particular, graphene oxide was used both as scaffold for peptide nucleic acid and as a quencher for the fluorophore attached to the PNA probe. After the addition of the target, the labelled PNA was able to emit fluorescence in a concentration-dependent manner monitoring target microRNAs in the picomolar range (Scheme 5.2).



Scheme 5.2 Strategy for the sensor based on graphene and PNA for multiplexed miRNA sensing in vitro. The fluorescence signal gets recovered when the fluorescent dye-labelled probes, initially adsorbed onto the surface of graphene, detach from graphene and hybridize with the target miRNA. (Reprinted with permission from Ryoo et al. (2013). Copyright (2013) American Chemical Society)

2.2 Metal Nanoparticles

Metal nanoparticles (MNPs) are a group of functional materials with distinctive chemical and physical characteristics, which are closely dependent on their shape, structure, composition and size. Great advances have been attained in MNP synthesis and applications in numerous fields such as electronics, sensors, catalysis and medicine (Han et al. 2017; Prasad et al. 2016). For this reason, MNPs have received a huge interest in the realization of biosensors for biomedical fields. Gold nanoparticles (AuNPs) in biology and nanomedicine have made significant progress over the past decade, particularly in therapeutic and imaging applications (Lv et al. 2018). These skills are based on their characteristic properties including finely adjustable optical properties, high specific surface area and the possibility of modifying the surface. AuNPs have been used as catalysts in many biomedical applications. In a further development, gold nanoparticles are active in redox, which opens up the possibility of miniaturizing detection devices at the nanoscale, offering excellent prospects for chemical and biological detection.

Gold nanoparticles have found significant exploitation in the biomedical field, thanks to (i) their comparative chemical stability, which makes them less hazardous; (ii) their simple and direct synthesis and manufacturing process; and (ii) their actual biocompatibility and non-interference with other labelled biomaterials (e.g. antibodies and other biomarkers) (Ho et al. 2010). Hu et al. developed a nanoporous gold electrode modified with encoded multifunctional gold nanoparticles to fabricate an electrochemical DNA sensor (Hu et al. 2008), describing the ability of gold nanoparticles to amplify the detection signal efficiently and detect DNA targets quantitatively, in the range of 8.0×10^{-17}–1.6×10^{-12} M.

Silver nanoparticles (AgNPs) also showed their suitability for biosensing approaches, being capable of providing high conductivity and biocompatibility. Indeed, highly sensitive and selective sensors based on noble silver nanoparticles created the possibility of developing new diagnostic platforms for disease markers, biological and infectious agents in the early detection of diseases and other physiological threats (Chen and Chatterjee 2013). As an example, AgNPs were electrodeposited on a glassy carbon electrode modified with a poly(ferrocenylsilane) DNA network to fabricate a hydrogen peroxide sensor. The electrochemical experiments demonstrated that this sensor had a high catalytic ability in the reduction of H_2O_2. This sensor provided a linear range of 2.0 mM to 353 mM with a detection limit of 0.6 mM, becoming very useful to reveal H_2O_2.

Thanks to the size- and shape-dependent optoelectronic properties, platinum nanoparticles (PtNPs) are similarly appealing materials for a wide biomedical application. The use of nanoparticles in biology leverages the dimensional and functional characteristics of their surfaces, as well as their inorganic nucleus, which results in specific physical properties. Furthermore, the size of these nanomaterials provides a large surface-to-surface-volume ratio as particle size decreases; the population of surface atoms increases significantly (Chen and Chatterjee 2013). By exploiting these outstanding properties, therapeutic nanoparticles could provide feasible alternative platforms for treating a great assortment of human diseases in clinical settings.

2.3 Titanium Dioxide Nanotubes

Titanium dioxide (TiO_2) nanotubes have sparked significant research activity thanks to their unique properties, as well as their simplicity of preparation, high orientation, large surface area, high uniformity and excellent biocompatibility. As a renowned semiconductor, nanostructured TiO_2 has also attracted a lot of attention due to its non-toxicity, long-term stability, low cost and multifunctionality (Zixue Su 2011). TiO_2 nanotube modification with other nanomaterials has been also reported to additionally improve the sensor performances. As an example, gold nanoparticles were electroplated on TiO_2 nanotube arrays and used to detect ascorbic acid (Babu et al. 2012). The sensor was analysed following its morphological and electrochemical characteristics and tested with ascorbic acid and other biomolecules with very promising results. The sensor was also challenged for ascorbic acid analysis in lemon juice exhibiting a sensitivity of 63.91 mA mM^{-1} cm^{-2} and admirable selectivity towards ascorbic acid in the presence of uric acid, dopamine, glucose and para-acetaminophen as interferents.

2.4 Zinc Oxide Nanoparticles

Zinc oxide (ZnO) exhibits a widespread variability of nanostructures with unique semiconducting, optical and piezoelectric properties. The surface of ZnO possesses several -OH groups, excellent candidates for the functionalization by molecules decorating different surface. Indeed, zinc oxide nanoparticles (ZnONPs) are used worldwide, which have attracted attention towards research on their impact on human health. In addition, many studies described the use of zinc oxide nanoparticles in the biomedical sector mainly focused on cancer cell imaging applications. They have been positively employed in various biomedical applications as gene transmission and biosensors (Zhang et al. 2014).

Several studies have established that zinc oxide nanoparticles are toxic to different bacterial species such as *Escherichia coli* and *Staphylococcus aureus*, as well as to primary human immune cells. A concentration > 3.4 mM and > 1 mM resulted in complete growth inhibition of *E. coli* and *S. aureus*, respectively (Rajeshkumar et al. 2019).

3 Immobilization Strategies at the Nanoscale

Biosensors have been considered economical and functional analytical devices broadly used in the last years, owing to their fast and simple use in the determination of specific analytes, particularly, and in site complex samples. In the biosensor field, recent progress on nanomaterials has headed to a dramatic development in the miniaturization of sensing devices, with reduced sample volume and manufacturing

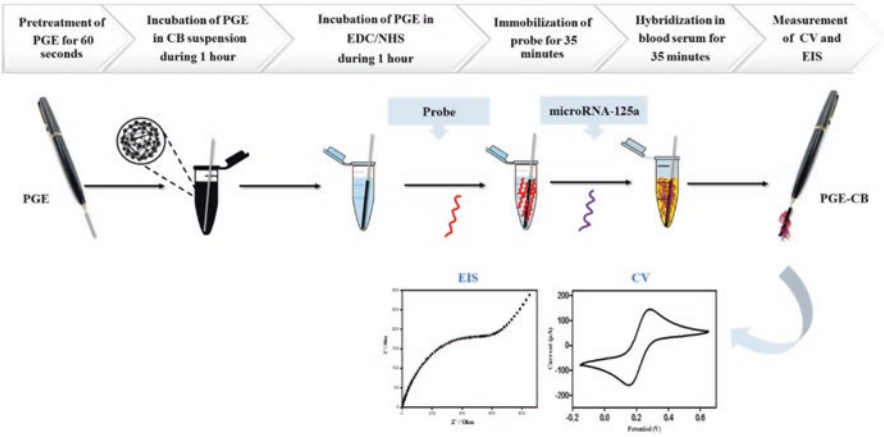
costs. In addition, since biological processes occur at the nano- and microscale, nanostructured materials have shown an exceptional platform to induce better interaction between biological species and the sensor surfaces, which guarantees higher stability and sensitivity of the bioelement. Thanks to their high specific surface area, nanoparticles can strongly adsorb biomolecules, thus showing their great potential in immobilizing biomolecules for the assembly of biosensors. The arrangement of nanomaterials and various biomolecules, as enzymes, nucleotides, antigens, DNA and amino acids, allowed the fabrication of several miniaturized and nanostructured devices and also implantable biosensors for real-time analysis, being nanomaterials capable of preserving their bioactivity.

As most of nanoparticles are electrostatically charged, they can immobilize biomolecules by electrostatic interactions. For instance, gold nanoparticles can immobilize proteins by covalent bonds formed between gold atoms and amine and thiol groups of proteins. Indeed, gold nanoparticles are frequently deployed for protein immobilization (Pingarrón et al. 2008). At the beginning of the 1990s, Zhao et al. (1996) immobilized several types of enzymes with AuNPs and manufactured different enzyme electrodes, and the enzyme electrodes prepared preserved their enzymatic activity for long period.

SiO_2 nanoparticles are also an outstanding matrix for enzyme immobilization owing to their good biocompatibility and simplicity of preparation. Further nanoparticles, including Pt, Ag, TiO_2 and ZrO_2, among others, can likewise be exploited to immobilize enzymes.

In order to modify the electrode of an immunosensor, several nanomaterials can be used for the immobilization of the bioelement and, at the same time, provide the amplification of the signals. Among nanomaterials, graphene is a talented nanomaterial in the biomedical field due to its excellent conductivity, large specific surface, good resistance and high biocompatibility (Bolotin et al. 2008). Fan et al. (2019) developed an electrochemical immunosensor based on rGO/Thi/AuNPs nanocomposites to determine CA125, which displayed an extensive linear range and low detection limit, with high sensitivity and accuracy. An unlabelled immunosensor for Japanese encephalitis B vaccine was prepared by immobilizing related antibodies with gold nanoparticles (Channon et al. 2018).

A further kind of biomolecule, DNA, is also immobilized with nanoparticles and applied for the realization of electrochemical DNA sensors. Various nanomaterials have been employed as platform for microRNA determination, as carbon black (CB) (Yammouri et al. 2017) and AuNPs (Mandli et al. 2017). The first example describes the use of carbon black to modify pencil graphite electrodes, in combination with multi-walled carbon nanotubes and graphene oxide to fabricate a sensor platform for microRNA-125a detection (Scheme 5.3). The DNA probe was covalently immobilized into the surface of the nanomaterial-modified electrode, conferring robustness, reproducibility and sensitivity, with a limit of microRNA-125a detection of 10 pM (1 pg/mL) within a linear response between 0.008 and 15 μg/mL, which corresponds to 1 nM and 2 μM. This demonstrated the ability of nanomaterials to furnish a suitable environment for the bioreceptor immobilization and augmented signals, thanks to carbon black.



Scheme 5.3 Scheme of the pencil graphite electrodes modified with carbon black, multi-walled carbon nanotubes and graphene oxide nanomaterials for microRNA-125a hybridization and analysis. (Reprinted with permission from Yammouri et al. (2017). Copyright (2017) Elsevier)

Likewise, AuNPs offer excellent biocompatibility and conductivity and are able to immobilize thiolated bioelements easily by the strong Au-S bond (Suzuki et al. 2009). To report an example, Yammouri et al. constructed a biosensor by immobilizing microRNA-21 complementary thiolated capture probe labelled with methylene blue on the surface of a carbon black and gold nanoparticle-modified pencil graphite electrode, showing good selectivity, stability and reproducibility (Yammouri et al. 2017).

Hasanjani and Zarei (2019) developed an ultrasensitive electrochemical sensor for the determination of mercury (II) using deoxyribonucleic acid/poly-L-methionine-gold nanoparticles/pencil graphite electrode (DNA/PMET-AuNPs/PGE). Immobilization of biomolecules with nanoparticles may be an excellent option, as it can effectively increase stability and maintain molecular activity.

Tsai and co-workers (2009) immobilized glucose oxidase (GOx) on SWCNT, and it was reported that the enzyme retained 75% of its activity by adsorption. The resulting GOx-SWCNTs were used in electrochemical layer-by layer biosensors for glucose, demonstrating a good sensor response.

Rubianes and Rivas (2005) described a SWNT-mineral-oil paste containing lactate oxidase for amperometric lactate monitoring. Dependable lactate monitoring is essential for clinical diagnosis, sports medicine, biotechnology and food analysis. The accelerated electron transfer reaction from hydrogen peroxide to the SWNT paste electrode allowed rapid detection of the analyte at a low potential (0.10 V).

A sensitive biosensor for cholesterol was fabricated by immobilizing cholesterol oxidase on a MWNT-modified gold electrode, using a layer-by-layer adsorption technique (Guo et al. 2004). An ideal cholesterol sensor should have sensitivities in the range of 2.5–10 mM since a total blood cholesterol level of less than 5 mM is considered to be risk-free, whereas high cholesterol levels of greater than 6 mM are

considered dangerous. The sensor response was linear in the range 0.2–6 mM, in agreement with the requirement for cholesterol concentration. In another case, a screen-printed electrode modified with MWNT mat and cholesterol oxidase had the capacity to detect cholesterol directly in the blood in clinically relevant ranges (Li et al. 2005). It was observed that carbon nanotubes promoted the electron transfer and almost doubled the sensitivity when compared to the control. Moreover, the carbon nanotube electrodes demonstrated a good correlation with the results of clinical analyses of blood samples from 31 patients.

4 Nanomaterials as Mediators

The electrical connection between the electrode surface and biomolecules represents a crucial parameter in the development of electrochemical biosensors (Hayat et al. 2014). It is noteworthy that conductive features of metal nanoparticles increase the electron transfer rate between the transducer surface and biomolecules. The mediators most commonly employed are replaced by metallic nanomaterials. The importance of these mediators has been described when projecting amperometric biosensors (Hayat et al. 2014). Metal nanoparticles function as electron or wire transfer mediators to supersede the mediator currently used in the design of electrochemical biosensors. In contrast, certain non-metallic nanoparticles as semiconductor and oxide nanoparticles may also enhance the electron transfer rate between proteins and electrodes. Silver particles have also a high electrical conductivity and can therefore improve the electron transfer. Furthermore, in conjugation with silver nanoparticles and pyrolytic graphite electrodes, they can serve as electrical bridges to wire the electron transfer between the electrode surface and biomolecules. In a similar context, Ghalkhani et al. (2009) investigated the electrocatalytic performance of electrodes modified with Pt nanoparticles (PtNPs) and two dendritic hyperbranched carbosilane polymers for the NADH oxidation. The suggested strategy allowed measuring NADH from +0.3 V (vs SCE), offering total protection against poisoning electrodes, using alcohol dehydrogenase (ADH).

Gold nanoparticles have been also deployed to enhance electron transfer between electrode surface and biomolecules in the development of enzymatic biosensors (Liu and Ju 2003). Wang et al. (2015) developed a novel MXene-Ti_3C_2 by etching Al from Ti_3AlC_2, to immobilize haemoglobin (Hb) and fabricate a mediator-free biosensor with an oxidized surface. Spectroscopic and electrochemical results validated that MXene-Ti_3C_2 had a strong enzyme immobilization with biocompatibility for redox protein, showing excellent bioactivity and stability of the proteins. Thanks to the peculiar structure of MXene-Ti_3C_2, the direct electron transfer of Hb was accelerated, and the developed biosensor showed brilliant ability for H_2O_2 detection with an outsized linear range of 0.1–260 μM and extremely low detection limit of 20 nM (based on a signal-to-noise ratio of 3). The immobilization of proteins onto the surface of MXene-Ti_3C_2 showed its ability as in the design of a new sensitive

and stable electrochemical biosensor for H_2O_2. In this context, the identification of H_2O_2 is extremely important because it plays an essential role in food, pharmaceutical and environmental analyses (Somasundrum et al. 1996). H_2O_2 can be measured at low applied potentials by using peroxidase as an electrocatalyst for electrochemical reduction. Amperometric assay combining mediators with peroxidase is one of the most sensitive procedures. However, the addition of the mediator increases the time and cost of the analysis. Hence, the co-adsorption of thionine and horseradish peroxidase on the electrode surface has been exploited to realize H_2O_2 sensors (Liu and Chen 2005), greatly simplifying the analysis system without the addition of a mediator to the solution.

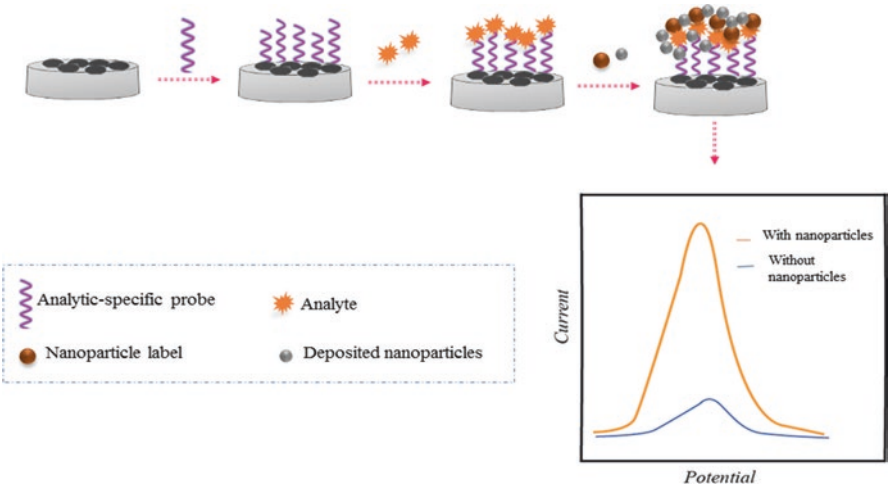
5 Nanomaterials as Signal Amplifiers

Several efforts have been made in the last decades to amplify the detectable signals of electrochemical biosensors (Miao et al. 2015). Consequently, several signal amplification approaches were investigated, such as enzymatic catalysed amplification (Akama et al. 2016), nucleic acid amplification technology (Zhao et al. 2015) and molecular conversion amplification (Yang et al. 2016). Alternatively, nanomaterial-based signal amplification has been widely used in the construction of biosensors owing to their large specific surface, catalytic property and biocompatibility (Chen and Chatterjee 2013). Initially, nanomaterials for signal amplification depend on their high specific area, where an increased amount of biomolecules can be loaded (Wang and Liu 2014). Recently, nanomaterials with catalytic activity are introduced into the design of electrochemical biosensor, in which nanomaterial can catalyse corresponding substrate to accelerate electron transfer for signal amplification (Voiry et al. 2013). Thanks to significant progress in nanosciences, electrochemical amplification of signals based on nanometric materials has excellent potential to enhance the sensitivity and selectivity of electrochemical biosensors (Mohammadi et al. 2019). The general procedures to configure electrochemical biosensors for target DNA/protein, where nanoparticle labels are used for signal amplification, are indicated in Scheme 5.4.

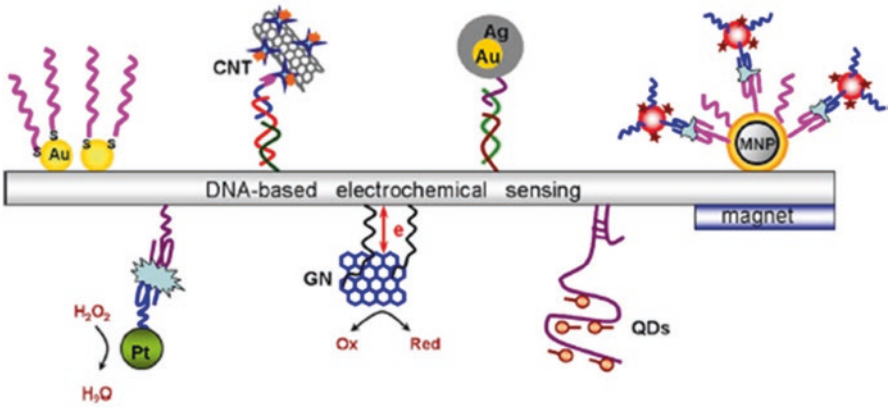
A variety of signal amplification pathways by nanomaterials are also outlined (Scheme 5.5), such as:

1. Electrode materials for constructing detection platforms.
2. Carriers for signal elements.
3. Tracers based on their direct electrochemistry.
4. Separators and collectors.
5. Catalysts.
6. Mediators to regulate the electron transfer process.

Finally several signal amplification techniques employing diverse nanomaterials and amplification procedures have also been demonstrated (Wu et al. 2014a).



Scheme 5.4 Schematic illustration of electrochemical affinity biosensors based on use of nanoparticles for signal amplification



Scheme 5.5 Schematic illustration of nanomaterial-based signal amplification strategies in DNA-based electrochemical sensing. (Reprinted with permission from Wu et al. (2014a). Nano Today. Copyright (2014) Elsevier)

Because of their exclusive assets, various nanomaterials have been applied as electrode materials to create detection platforms, such as gold nanoparticles (AuNPs), carbon nanotubes (CNT), graphene (G), polymer NPs and their nanocomposites (Pilehvar et al. 2014). Biofunctional NPs can have a synergistic effect on catalytic activity, conductivity and biocompatibility to speed up signal transduction, leading to lower detection limits at even zeptomolar concentrations.

Several signal amplification strategies based on functional nanomaterials, coupled with different electrochemical methods, have recently gained considerable interest towards the emergence of high-performance analytical devices for the

sensitive detection of trace analyte amounts, including DNA and microRNA assays in clinical and environmental applications (Chen and Chatterjee 2013).

CNTs can also provide a wide specific surface area to immobilize DNA molecules and considerably enhance the electrochemical features of sensors (Zeladaguilløn et al. 2009). The Korri-Youssoufi group (Miodek et al. 2013) designed an electrochemical aptasensor to reveal human cellular prions (PrPC) based on multiwalled carbon nanotubes (MWCNTs) modified with fourth-generation polyamidoamine dendrimers (MWCNTs-PAMAM G4); PAMAM G4 was covalently attached to MWCNT via an amide linkage, and a large amount of functional groups for covalent fixation of the ferrocenyl group (Fc) as redox markers were introduced, further enhancing the amount of charge from the aptamers for prion fixation. The binding of prions triggered a decrease in the redox current of Fc due to the perturbation of electron transfer caused by changes in the conformation of the surface layer. Thanks to its high surface area with lots of functional groups and the unique electrical properties, a very sensitive detection limit of 0.5 pM was achieved.

The incorporation of signal amplification strategy with a microfluidic immunosensor was also reported in literature, for multiplexed measurement of cancer biomarkers in serum patient samples (Wu et al. 2014b). The authors designed an electrochemical device consisting of eight individual lines modified by graphene nanomaterials, further used as probes to label antibodies extremely sensitive to a range of specific analytes. Wu and co-workers (2013) similarly improved their graphene-based microfluidic immune device by triggering a controlled amplification by polymerization on the immune device surface and achieved a self-calibrating system. This innovative strategy for signal amplification was claimed to improve electrochemical signal output significantly to minimize ambient condition impacts and achieve ultrasensitive detection.

6 Nanomaterials for Bioreceptor Labelling

Alternative exploitation of nanomaterials is labelling of biomolecules as antigen, antibodies and DNA, once again demonstrating their crucial role in the design of highly sensitive biosensors. Among others, carbon nanomaterials were exploited as label supports when incorporated in biosensors. In this case, besides being conjugated to the label, they were also modified with the detection antibody or antigen, according to the test format. These mixed conjugates have been incorporated into sandwich and competing configurations offering benefits such as more labels and electrochemical signal amplification (Joseph and Mustafa 2003). Tang et al. (2010) doped MWCNTs with nanosilica and HRP and used them as labels of the anti-staphylococcal enterotoxin B (SEB) antibodies in a sandwich-type immunosensor. The results confirmed a great correlation with the values obtained using a commercially available enzyme-linked immunosorbent assay (ELISA). Competition-type immunoassays and sensors have also been developed using carbon nanomaterials as immobilizers for bioreceptors and labels. Tian et al. (2014)

conjugated peroxidase-mimicking DNAzyme and microcystins (MC-LR) to SWCNTs. This multi-labelled MC-LR competed with free microcystin in solution for their binding to capture antibodies immobilized on SWCNTs previously adsorbed on the electrode. The measured current decreased linearly as the MC-LR concentration increased from 0.01 to 7.0 ng mL^{-1}. Detection limit value as low as 2.3 pg mL^{-1} was attained, thanks to the properties of SWCNT in terms of promotion of electron transfer between the electrolyte and the electrode and the capacity of fixation of multiple label enzymes.

Metal nanoparticle labels can be also used in both immunosensors and DNA sensors (Ding et al. 2013). Moreover, by using external magnetic fields, magnetic nanoparticles linked to biomolecules can eliminate mass transfer problems and play a useful role as biomolecular supports and separation tool in microfluidic devices. Bound to an appropriate antibody, these nanomaterials can be exploited for the labelling of molecules, structures or microorganisms to realize immunoassays in which the magnetic field produced by the magnetically labelled targets is sensed by means of sensitive magnetometers. Binding of antibody to target molecules or disease-causing organism is the basis of several tests. Antibodies labelled with magnetic nanoparticles give magnetic signals on exposure to a magnetic field. Antibodies bound to targets can thus be identified as unbound antibodies disperse in all directions and produce no net magnetic signal (Kewal 2005).

7 Nanomaterials to Project Microfluidics, Lab-on-a-Chip and Point-of-Care

Over recent years, progress in microfluidics and lab-on-a-chip technologies has provided exceptional chances for the application of nanomaterial production processes, thanks to the miniaturization of the fluid environment. Microfluidic (MfD) devices can be incorporated into laboratory functions and processes reduced to a miniaturized chip format known as a "lab-on-a-chip", furnishing several advantages compared to conventional techniques (Kewal 2005). There are numerous factors to consider when designing a microfluidic device, such as the choice of materials, the dimensions of the MfD devices and fluid control devices (e.g. pumps, valves and mixers). MfD systems operate in small liquid quantities (10^{-9} to 10^{-18} L) (Dhyani et al. 2015) and can be manufactured from a huge number of materials as glass, silicon, polymers and gels. Since 1985, when Unipath Inc. first commercialized the pregnancy test, which is still widely used, microfluidic biosensors have found enormous attention in the medical field, especially in the form of miniaturization "lab-on-chip" (LOC). The principal benefits of microfluidic biosensors are a low sample volume, minimally invasive methods for sample collection, laminar flow, reduced reagent consumption, short reaction time for analysis, multiple analyte detection, portable and a high surface-to-volume fluid ratio compared to other conventional biosensors (Gu et al. 2010).

Recently, nanoparticles have got huge attention in the fabrication of MfD for the miniaturization of the sensing devices and enhance their analytical performances (Sri et al. 2019). The miniaturization of sensors headed to the realization of several point-of-care diagnostics that could be deployed in the field. In the meantime, some of these systems combining microfluidics and nanomaterials have demonstrated multiplexed detection capability through the use of a device array with unique functionality and good reusability for the detection of each analyte. Moreover, the modification of transducer platforms with nanoparticles generates highly conductive surface interfaces that allow sensitive/catalytic detection of ionic, molecular and biomolecular analytes. These highly sensitive sensors are easy to incorporate and become good candidates for LOCs.

CNTs are employed in several devices as transducers, thanks to their high sensitivity, specificity, rapidity in analysis, low cost and ease of use (Sri et al. 2019). Biosensors based on CNTs have been enhanced in their portability, functionality, reliability and real-time diagnosis for point-of-care analysis. The use of CNTs in POC systems has been considered for the analysis of biological analytes such as DNA, glucose, proteins and viruses. POC tests based on CNT biosensors can be divided into three types including CNT-based lateral flow tests (LFA), CNT-based printed electrode, and CNT-based lab-on-a-chip (LOC) (Syedmoradi et al. 2017).

The modified CNT transducers have a larger surface area, which increases current density and provides an increased surface area for fixing biomolecules (Serp and Castillejos 2010). This can augment current stability as well as the sensitivity of the detector. The electrical behaviour of these platforms depends on the chirality of the CNT, the number of carbon layers, defects and their functionalization, which must be carefully studied during their application. All these CNT properties are useful for catalysis (Serp and Castillejos 2010), enzymatic immobilization (Feng and Ji 2011), protein detection (Zhang et al. 2011) and metal detection (Morton et al. 2009). The coupling of CNTs with LFA strips can deliver a reasonable, fast and sensitive approach for many biomolecule determination including DNA and protein. Recently, Qiu et al. (2015) developed a MWCNT-based lateral flow biosensor using streptavidin-biotinylated probe on nitrocellulose membrane and carboxylated MWCNT as a label. Amine-modified DNA detection probe was immobilized to the carboxylated MWCNT by covalent bonding between DNA amines and carboxylic acids of the CNT. This biosensor delivered a fast and sensitive DNA detection with a limit as low as 40 pM. In addition, this platform exhibited high reproducibility in the absence and presence of 5.0 nM and 50 nM target DNA. Combining lateral flow with the exclusive physical properties of MWCNTs, a 12.5 times enhanced sensitivity was achieved if compared to the GNP-based lateral flow.

A CNT-SPE has been employed for the electrochemical detection of human chorionic gonadotropin (hCG). The CNT working electrode was modified with aminopropyl triethoxysilane (APTES), in order to introduce amino groups onto CNT surface. These amino-modified CNTs were then attached to the antibody-target hCG by use of the cross-linking agents EDS and NHS. This modified electrode showed a high specificity, wide linear range (0.01 × 10–9–100 × 10–9 g cm-3) and

low detection limit for hCG. The excellent detection capability and simplicity of this approach makes it a convenient method for detecting hCG in a POC diagnostic.

Okuno et al. have designed an unlabelled immunosensor modified with CNTs for the detection of total prostate-specific antigen. It should be noted that CNTs provide better electron transfer, obtaining an improved detection limit of 0.25 ng mL^{-1} if compared to unmodified electrodes.

Chua et al. (2011) reported a graphene oxide-based microfluidic amperometric detector that showed higher peak sensitivity, resolution and separation efficacy than the same device without modification. Fibronectin detection was provided with a detection limit of 0.5 nM, highlighting its ability to be considered as proof of concept. Finally, nanoelectrodes and nanostructured electrodes attract attention for the analysis of biological samples at very low concentrations where the reduction in sensor size seems to facilitate the detection of reaction products on a similar scale.

8 Nanoelectronics

Nanoelectronics requires the development of devices at the nanoscale, to focus on low-power consumption, compactness and high memory chip (Gopinath et al. 2013b). Recently, researchers in the field of nanoelectronics have started to focus more on the application of nanobiosensors for early detection of diseases, their treatment and prevention (Gopinath et al. 2013a). High-performance detection tools have been projected to achieve two vital goals: (i) generating suitably high sensitivity electronics and (ii) providing compatibility with the bioelements (Huang and Chen 2010).

Specifically, different nanoelectronic biosensors have been successfully deployed from carbon nanotubes (Brett et al. 2007), nanowires (Patolsky et al. 2006), nanopores (Howorka and Siwy 2009) and recently graphene (Agarwal et al. 2010). Compared to conventional optical, biochemical and biophysical methods, electronic biosensing based on nanomaterials offers unique benefits, as high sensitivity and new sensor capabilities, high spatial resolution for localized detection, compatibility with miniaturized lab-on-a-chip systems and easy integration with recording electronics for real-time monitoring at high time resolution and simple, non-invasive detection without the need of labelling (Huang and Chen 2010).

Among the different electrical biosensor structures, devices based on field-effect transistors (FETs) have attracted much attention because they can directly translate the interactions between target biological molecules and the FET surface into readable electrical signals (Chen et al. 2011). Silicon nanowire field-effect transistors (SiNW-FETs) have recently gained huge consideration as a promising tool in biosensor design due to their ultrasensitivity, selectivity and real-time, label-free detection capabilities towards proteins, DNA sequences, small molecules, cancer biomarkers and viruses (Chen et al. 2011). Specific peptide nucleic acid (PNA)-modified SiNW-FET sensors have recently been established to diagnose dengue virus infection (Zhang et al. 2010). Synthetic PNA receptors were first anchored to

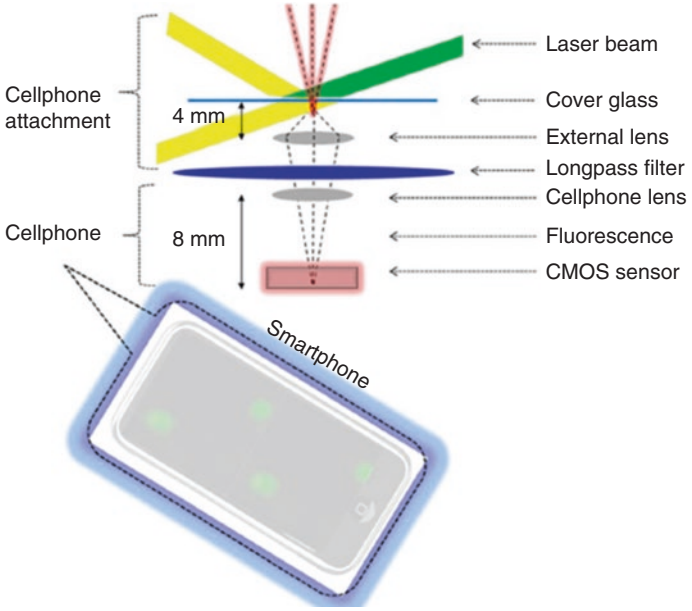
a SiNW-FET surface. A specific fragment (69 bp) derived from dengue serotype 2 (DEN-2) virus genome sequences was selected as the target DNA and amplified by the reverse transcription-polymerase chain reaction (RT-PCR). Distinctive resistance changes between the two different PNA receptors (i.e. complementary and non-complementary to the target DNAs) can be distinguished. The detection limit of this biosensor based on SiNW-FET was claimed to be 10 fM. These investigations suggested that the PNA-modified SiNW-FET sensor incorporated with RT-PCR has been successfully developed for a rapid and ultrasensitive diagnostic method of detecting dengue virus.

A similar promising approach allowed for revealing microRNAs (miRNAs) in early diagnosis of cancer (Zhang et al. 2009a). miRNAs have been characterized to play an important role in cell development and to be related to a number of cancers and neurological disorders. Therefore, the detection of miRNAs becomes more and more important in the field of medical science. A PNA-immobilized SiNW-FET was used to probe miRNA by detecting PNA-miRNA hybridization via base pairing; this approach displayed an admirable detecting specificity in discriminating a single-base mismatch in miRNA. Moreover, the application of a PNA-functionalized SiNW-FET to probe the hybridization with complementary miRNAs is obviously preferential to a DNA-functionalized SiNW-FET, again indicating that neutral PNA prefers to hybridize miRNAs. Also, the PNA-functionalized SiNW-FET sensor was capable to sense a specific miRNA in total RNA extracted from HeLa cells.

Interdigitated electrode (IDE)-based sensors have been also described, consisting of multiple electrodes and acting as capacitive sensors (Hong 2012). IDE can be embedded with a suitable thin film, which facilitates performance of surface functionalization for biosensing applications (Choi et al. 2010). The silver IDE electrode is a very suitable material, due to good conductivity and ease of deposit on the silicon wafer sample by a conventional wet etching method. Exposure to ultraviolet (UV) light allows the pattern transfer to be done from the IDE mask fitted on the surface of the sample. A coupled IDE may apply for typing and subtyping of influenza viruses (Gopinath et al. 2012, 2013a).

Zhu et al. (2012) developed a cost-effective bacterial detection device that can be labelled on a cell phone. This device is the first cell phone-based imaging system for the observation of a single bacterium or virus and has demonstrated the detection of *E. coli* as proof of concept. With a detection limit of 5–10 CFU/mL, this handheld device was shown to offer higher specificity for detecting *E. coli*, even considering the samples, which included a complex food matrix (Scheme 5.6).

A few decades later, Varma et al. (2005) described the use of an optical biosensor on rotary disc interferometry for very high-throughput immunoassays (antibody-based), called BioCD. The BioCD works like an analogue sensor but is attached to antibodies to replace digital recording. The BioCD operates according to the micro-diffraction quadrature principle, which allows sensitive linear detection of analyte and ligand interactions. Using rotary disc interferometers, an immobilized specific complexed analyte/ligand can be recognized using higher speed and sensitivity. This system has been beneficial for the analysis of *E. coli*. Similarly, a wide-ranging variety of bacterial species can be detected on disc technology using an appropriate probe.



Scheme 5.6 Smartphone-based detection. An electronic-based device for point-of-care applications. (Reprinted with permission from Gopinath et al. (2019). Copyright (2019) Elsevier)

9 Nanoparticle Toxicity

Nanotechnology is increasing rapidly with nanoparticles produced and used in a wide range of commercial products worldwide. For instance, silver nanoparticles (AgNPs) are widely employed in electronics, biosensing, clothing, food industry, cosmetics and medical diagnostics. Nevertheless, these widespread applications increase human exposure and therefore the potential risk associated to their short- and long-term toxicity. Intravenous injection of Ag nanoparticles has recently been evaluated for drug administration and targeted applications. Rosas-hernández et al. (2009) investigated if Ag nanoparticles induced selective and specific biological effects on coronary endothelial cells (CEC) and regulated vascular tone in aortic rings isolated from rats. At low concentrations, Ag nanoparticles function as antiproliferative/vasoconstrictor factors that interfere with nitric oxide (NO) production. At high concentration, Ag NP stimulated no meditated proliferation/vasorelaxation. This study showed that the level of exposure to Ag nanoparticles played a crucial role in toxicity and could have other physicochemical effects. A major concern with graphene-based materials is that knowledge of their environmental toxicity and biological safety profile is limited. The British government agency, the Medicines and Healthcare Products Regulatory Agency (MHRA), and the US Food and Drug Administration (FDA) are currently reviewing all forms of graphene and functionalized graphene oxide (GO) because of their low solubility, high agglomeration, prolonged retention and relatively long circulation time in the blood (Nezakati et al. 2014).

The interactions of AuNPs with biological systems are often associated with their physicochemical characteristics that allow them to be absorbed into cells, which is not possible for larger particles. This is one of the reasons why AuNPs can be toxic compared to larger particles when compared to a massive dose (Fanord et al. 2011).

Some studies of cytotoxicity were demonstrated in triphenylphosphine-stabilized AuNPs employing four cell lines like tissue fibroblasts (L929), epithelial cells (HeLa), macrophages (J774A1) and melanoma cells (SK-Mel-28) (Pan et al. 2007). The results of these studies demonstrated that cellular response is dependent on size. As an example, 1.4 nm AuNPs were observed as the most toxic substance responsible for rapid cell death by necrosis, compared to 15 nm which was found to be non-toxic (Chen et al. 2009). However, cell proliferation was inhibited, which was linked to negative regulation of cell cycle genes. In addition, oxidative DNA damage was found in conjunction with decreased regulation of DNA repair (Coradeghini et al. 2013).

However, as stated by Antonacci et al. (Antonacci and Scognamiglio 2019), "several criticisms emerged regarding the use of nanomaterials. The main issue is related to their most important aspect, the nano-size. Indeed, while this feature determines high reactivity and great capacity, it could become potential lethal factor by inducing adverse cellular toxic and harmful effects".

For this reason, a main further concern should regard the implementation of the toxicological effects of nanomaterials on animal and plant cells, since few data are nowadays available and further investigations would be strongly required.

10 Green Nanomaterials

In recent years, nanomaterials have been intended to operate as intelligent and multifunctional materials in medicine and pharmacy, in particular in the diagnosis and treatment of cancer, as nanostructured electrodes in batteries and single-walled carbon nanotubes in communication technology devices, but also as antimicrobial materials in the cosmetics, food and clothing industries (Chithrani et al. 2006). Although nanomaterials have many applications and advantages, their production and applications are expensive and in some cases accompanied by the creation of environmentally harmful by-products (Lim et al. 2009).

Moreover, the application of synthetic nanostructures in medicine is limited due to their risks and side effects. That is why, nowadays, scientists are trying to use green ways to synthesize nanomaterials to prevent such side effects. In fact, green nanotechnology refers to the use of nanotechnology to improve environmental sustainability by means of green processes to minimize the potential environmental costs and risks associated with the negative externalities produced (Nair and Pradeep 2002). Green nanotechnology represents a new route inspired by the ability of nature to eliminate or reduce the impact of nanomaterials on the ecosystems and the human well-being; this can encourage the replacement of existing nanomaterials with newer more environmentally friendly ones.

Table 5.2 Synthesis of nanomaterial from some green substrates

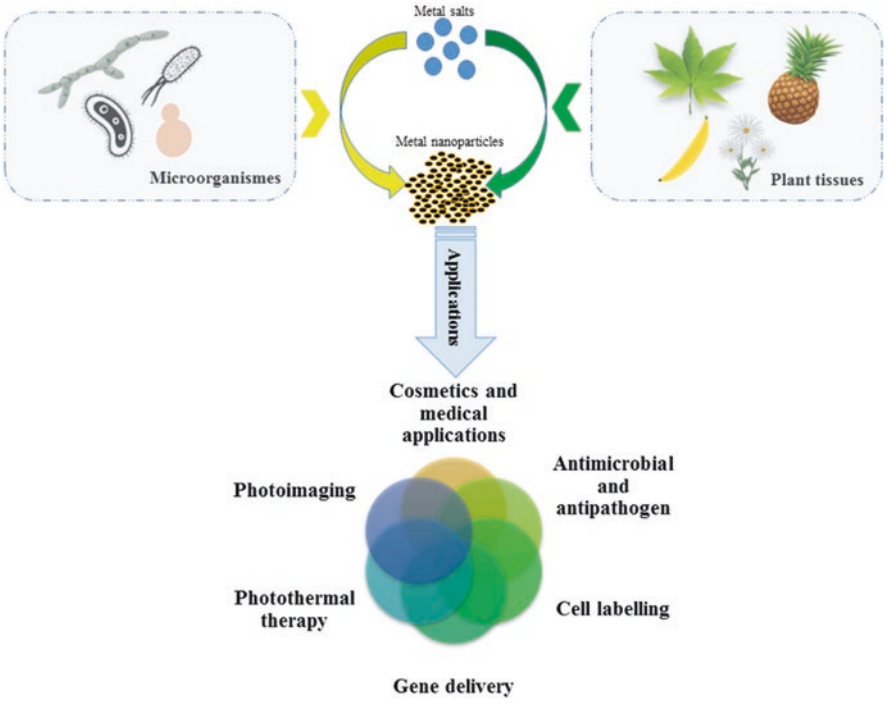
Green substrates	Name	Type of nanomaterial	Size range (nm)	Ref.
Plant	*Cinnamomum camphora*	Au and ag	55–80	Huang et al. (2007)
	Aloe vera	Au	50–350	Chandran et al. (2006)
	Alfalfa sprouts	Ag	2–20	(Gardea-torresdey et al. 2003)
	Avena sativa (oat)	Au	5–85	(Armendariz et al. 2004)
Algae	Marine macroalgae			
	Caulerpa peltata	Au	9–20	Xie et al. (2007)
	Hypnea valencia	Au	8–12	Singaravelu et al. (2007)
	Chlorella vulgaris			
	Sargassum wightii			
Fungi	*Phoma* sp. *3.2883*	Ag	71.06–74.46	Chen et al. (2003)
	Fusarium oxysporum	Au	20–40	Ahmad et al. (2003)
	Aspergillus fumigatus	Ag	5–25	Bhainsa and Souza (2006)
Bacteria	*Pseudomonas stutzeri*	Ag	1–20	Wang et al. (2018)
	Acinetobacter spp.	Ag	10	Nadhe et al. (2019)
	Escherichia coli DH5α	AU	25–33	Du and Wang (2016)

Nature has developed various processes for the synthesis of inorganic materials at the nanoscale and microlengths, which have contributed to the development of relatively new products, and a largely unexplored field of research is nowadays based on nanomaterial biosynthesis (Mohanpuria et al. 2008). The microbial enzymes or plant phytochemicals with anti-oxidizing or reducing properties are usually responsible for metallic compounds in their respective nanoparticles. A lot of nanomaterials are synthesized from different substances, such as plants, algae, fungi and bacteria (Table 5.2).

Recently, the diverse applications of metal nanoparticles produced by biological synthesis have been explored in biomedical, agricultural and environmental areas (Scheme 5.7).

Since ancient times, plants have been used as natural remedies to cure many physiological disorders in traditional oriental medicine, particularly in India and China. The “green” synthesis of copper (cu), gold (Au), Nickel (Ni), platinum (Pt), titanium (Ti), selenium (Se), silver (Ag) and zinc (Zi) nanoparticles (NP) using plant resources had already been reported in the literature (Mirzaei and Darroudi 2017).

The manufacture of AuNPs using plants as a natural source has resulted in a better-quality, more environmentally friendly product (Chandran et al. 2006). For example, the plant extract of *Aloe vera* was used to obtain gold nanotriangles



Scheme 5.7 Biological synthesis and biomedical applications of metal nanoparticles

with a size between 20 and 50 nm (Das et al. 2011). Different AuNP syntheses have been described using a variety of plant sources and obtaining various shapes. AuNPs synthesized by plants are more stable than those synthesized by other methods.

Au, as a noble metal, was used in prehistoric times in ancient cultures such as China, Egypt and India to cure a variety of diseases such as measles, smallpox, syphilis and skin ulcers. Currently, Au is used as a stent, pacemaker and middle ear implant and in dental restoration as alloys (Svedman et al. 2006).

Copper oxide (CuO) nanoparticles have been also reported for their antimicrobial activity against infectious organisms such as *E. coli*, *Bacillus subtilis*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Syphilis typhus* and *Staphylococcus aureus* (Akhavan and Ghaderi 2010; Stoimenov et al. 2002). Materials from plants including magnolia leaf extract and *Euphorbia nivulia* stem latex have been employed to synthesize Cu nanoparticles, which have also been used as non-toxic aqueous formulations for the administration of cancer treatments (Padil and Cernik 2013).

Abboud et al. (2014) used brown alga (*Bifurcaria bifurcata*) in the biosynthesis of copper oxide nanoparticles of dimensions 5–45 nm. The synthesized nanomaterial is characterized by UV-visible absorption spectroscopy and Fourier transform infrared spectrum analysis. X-ray diffraction confirms the formation and crystalline nature of copper oxide nanomaterial. Moreover, these nanoparticles showed high antibacterial activity against two different strains of bacteria, *Enterobacter aerogenes* (Gram negative) and *Staphylococcus aureus* (Gram positive).

Significant interest has arisen in the research of NPs during the last decades, regarding biomedical applications in particular. The integration of nanotechnologies into medical science has opened up new opportunities and led to a better understanding of molecular biology. As a result, it is possible to offer new methods for the treatment of diseases that were previously difficult to target due to size restrictions (Salata 2004). The synthesis of biofunctional nanoparticles is very important for biomedical applications and has recently attracted the attention of many research groups that are constantly evolving in this field (Zhang et al. 2008).

A study by Yuan et al. (2010) used ZnO quantum dots as a drug delivery system to target doxorubicin in HeLa cells. They encapsulated ZnO nanoparticles with chitosan to improve the stability of nanomaterials. Their results indicated that this drug delivery system could be used as an effective way to improve patient quality of life system for targeting doxorubicin on cancer cells. Another main aspect in the application of nanoparticles is their use as vectors for gene transfer to different cells, particularly tumour cells (Taylor and Webster 2011). The use of this system for gene delivery is associated with various advantages. For example, the expression of plasmid containing gene on NP surface could ensure safe and efficient gene targeting to the receipt tissues (Asharani et al. 2008).

As with plants, microorganism would be used as a "bio-factory" for the synthesis of metallic nanoparticles, and a set of biological protocols for the synthesis of nanoparticles has been reported using bacterial biomass, supernatants and derived components.

Besides the use in the bioclinical field of green nanoparticles as drug delivery systems or for cancer treatment, their exploitation for the design of nanosensors is gaining momentum. A fervent literature is emerging in this field for the detection of bioclinical markers as glucose (Atchudan, 2019), alcohol (Gayda et al. 2019) and lactose (Bollella et al. 2017).

11 Conclusions

The medical diagnosis remains a primary focus in healthcare and personalized medicine, and the exploitation of biosensors aims to enable continuous monitoring of diseases as well as their follow-up. New diagnostic biosensors will help better diagnose and follow up diseases and treatments, and the implementation of novel POCs, easily usable by patients, and portable and implantable devices represents the challenge of the near future. In this scenario, nanomaterials have become important components in the design of bioanalytical devices because they are able to significantly improve diagnostic performance in terms of sensitivity, selectivity and robustness.

Furthermore, one of the main limitations of current nanotechnology is represented by the enormous financial costs associated with manufacturing and processing of nanoproducts. To this aim, further efforts should be achieved towards the synthesis of green nanomaterials by means of novel sustainable routes with reduced costs and energy consumption as well as endangering impact on the environment.

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Chapter 6
Stimuli-Responsive Nano-Drug Delivery Systems for Cancer Therapy

Sauraj, Anuj Kumar, Bijender Kumar, Ruchir Priyadarshi, Chhavi Sharma, Anurag Kulshreshtha, and Yuvraj Singh Negi

Contents

1 Introduction

Globally cancer is one of the leading causes of mortality in the world after cardiological diseases (Liu et al. 2017; Nagai and Kim 2017; Wang and Tian 2017). It can occur at any stage of life. Among the various cancer treatment, chemotherapy is most widely used to treat the various types of cancer becouse of its high cancer cell

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killing efficiency (Gao et al. 2017). Although several chemotherapeutic agents have been extensively used in cancer therapy but they are still far away from perfect because of their short half-life, rapid metabolism and low therapeutic efficacy (Kumar et al. 2017). However, unlike surgery and radiation therapy which targets specific areas, conventional chemotherapy works throughout the body and kills the cancer cells in addition to normal cells, thus causing adverse side effects (Zeng et al. 2017). Thus to reverse the serious side effects and further improve the therapeutic efficacy of chemotherapeutic drugs, various nano-sized systems like dendrimers, micelles, polymeric nanoparticles, nanoshells, etc. have been developed and been used as a carrier for the delivery of chemotherapeutic agents, due to their unique physiological characteristics (Lale et al. 2015; Sauraj et al. 2018). Although nano-drug delivery systems are capable to take the drug inside the cancer cells through EPR effect, complete release of drugs inside the cancer cells is an important issue in cancer drug delivery (Sauraj et al. 2018; Yang et al. 2015). Thus to solve this issue, a variety of stimuli-responsive systems that are capable to release the payload at the desire site of human body have received great attention (Li et al. 2013; Song et al. 2016; Zhao et al. 2017). Herein, the current progress of stimuli-responsive drug delivery systems and their application in biomedical application especially in cancer drug delivery as a carrier have been discussed in details.

2 Stimuli-Used for Trigger Drug Release

In recent years, a variety of nano-drug delivery systems that response to local environment of tumour tissue have been developed for cancer targeted drug delivery applications (El-Sawy et al. 2018). These biomaterials have been developed on the basis of internal or external stimuli of the tumour (Hossen et al. 2019; Raza, Rasheed et al. 2019). The internal stimuli that make different from normal tissues include local changes of different intensity in pH, temperature, redox conditions and the expression of certain biologically/enzymatically active molecules. External stimuli include magnetic field, heat, light (including laser beams) and ultrasound (Fig. 6.1) (Li et al. 2017).

3 Internal Stimuli-Responsive Drug Delivery Systems

3.1 pH-Responsive Drug Delivery System

There is a significant change in pH of our body parts, for example, the pH of the stomach is acidic (1.2–30) and the pH of the small intestine and large intestine are 7.0–7.4 and 6.5–70, respectively.

Moreover, the pH of human blood (7.4) and extracellular environment of tumour cells (6.5–7.0) is different from the pH of early endosomes (5.5–6.3) and the pH of

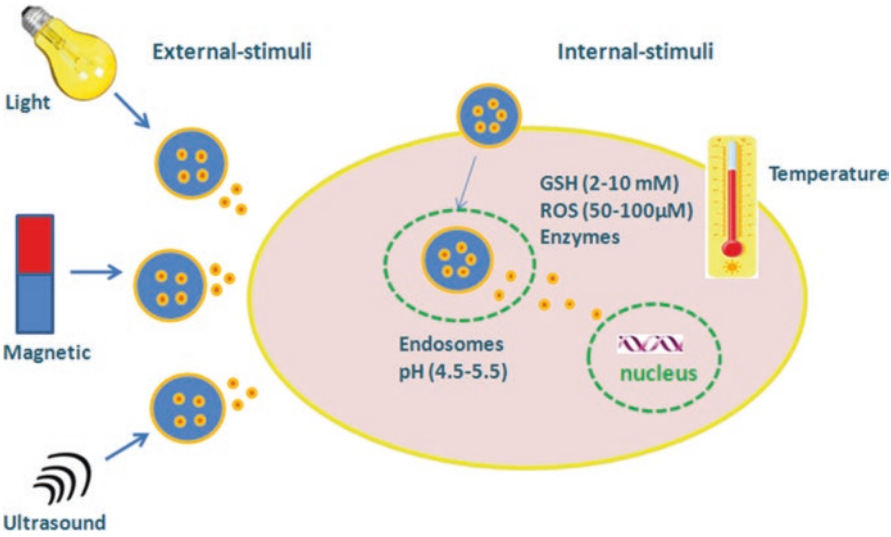

Fig. 6.1 Illustration of stimuli responsiveness utilized in cancer targeted drug delivery

late lysosomes (pH <5.5) (Zhou et al. 2018). On the basis of significant difference in the pH of intracellular and extracellular environment of tumour tissue and cell, various kinds of systems have been prepared and utilized for the intracellular delivery of chemotherapeutic agents in cancer therapy. The various types of chemical functionalities such as hydrazone, orthoester and acetal have been utilized to develop the pH-responsive systems (Badeau and DeForest 2019). The chemical structure of the most commonly used pH-responsive linkage is shown in Fig. 6.2. These pH-responsive bonds remain stable in the extracellular environments and degrade in the acidic environments of lysosomes, thus releasing the drug within cells.

By using the different acid-labile bonds between the polymer and drug, different kinds of pH-responsive drug delivery systems were prepared. In these system, anticancer drug is conjuated with the carrier molecule through the acid-labile linkage which formed self-assembled nanostructures in aqueous medium and remains inactive until the linker is hydrolysed.

The anti-cancer drug which has primary amine group such as doxorubicin (DOX) is generally attached to the polymer or carrier molecules through amide, hydrazone and cis-aconityl acid linkage. For example, Du et al. prepared dual pH-responsive polymer-drug conjugates based on PPC-Hyd-DOX-DA, where DOX was attached to polymer via the pH-responsive hydrazine bond. These nanoparticles showed acid-triggered drug release behaviour, and most of the drug was released in acidic environment. Further the cell cytotoxicity results demonstrate the enhanced therapeutic effect compared to free drug (Du et al. 2011).

In another study, Gao et al. (2017) prepared pH-responsive prodrug nanoparticles for the dual delivery of doxorubicin and curcumin. In this system, DOX was chemically linked with oxidized sodium alginate via a Schiff base reaction which

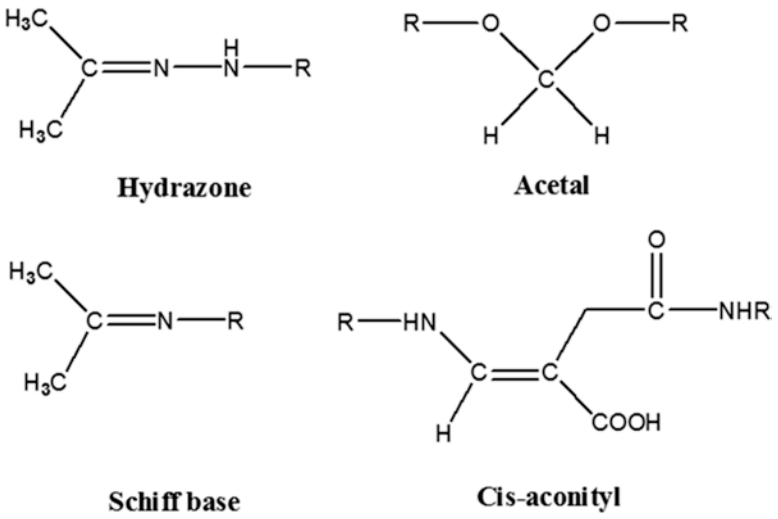

Fig. 6.2 The most commonly used pH-responsive chemical bonds

formed self-assembled behaviour in aqueous medium. Subsequently, curcumin was loaded into the self-assembled nanoparticles. The drug release study indicates that the curcumin-DOX NPs exhibited pH-responsive behaviour in acidic media and also demonstrated a remarkable efficacy towards MCF-7 cell lines.

Kakinoki et al. (2008) synthesize polyvinyl alcohol (PVA)-DOX conjugates through pH-responsive bond to improve the anti-tumour activity of DOX in cancer therapy. The drug release study demonstrated that the DOX was released from the conjugates after the hydrolysing the cis-aconityl bond.

3.2 Redox-Responsive Drug Delivery System

The presence of glutathione (GSH) in intracellular environments is much higher (0.5–10 mM) in comparison to extracellular environments (2–20 μM) (Badeau and DeForest 2019; Guo et al. 2018). On the basis of these differences in redox environments, a variety of redox-responsive systems have been prepared using various redox-responsive bonds. These stimuli-responsive bonds are often stable in normal tissues and degrade at the reductive environment of tumour cell and release activated drugs. The various types of chemical functionalities such as 3,3'-dithoidisulphide acid, 2-hydroxyethyl sulphide, cystamine and cystine have been used to design redox-responsive systems. The chemical structure of the most commonly used redox-responsive linkage is shown in Fig. 6.3. It is expected that these chemical bonds remain stable in the extracellular environments and cleaved in the specific environment of tumour cell.

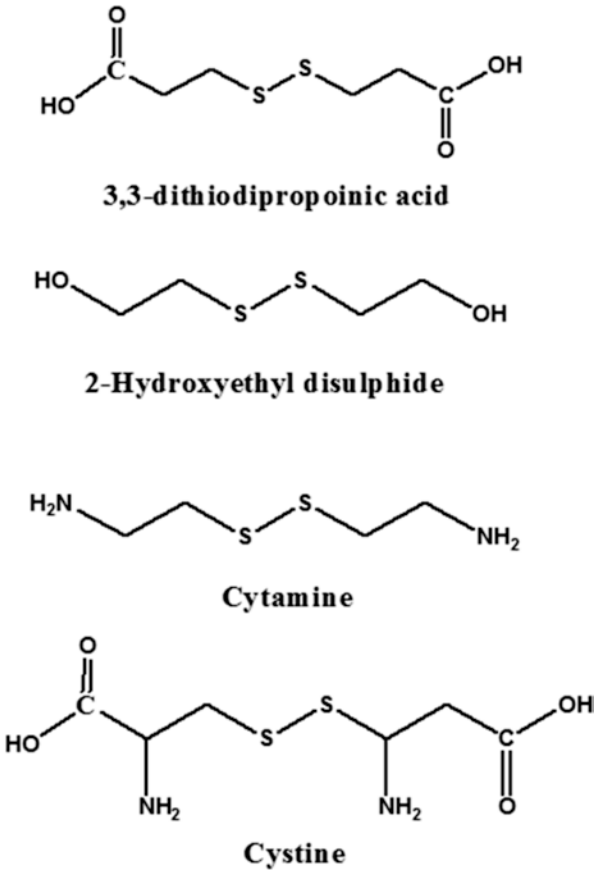

Fig. 6.3 The chemical structure of the most commonly used redox-responsive chemical bonds

Disulphide bonds are often used in development of stimuli-responsive delivery systems, which rapidly degrade by the GSH presence in cancer cells. These specific bond can be linked with polymer chain or drug molecules that further attach to them. In this regard, Guo et al. (2016) developed self-assembled polycurcumin nanoparticle through the conjugation of curcumin with PEG polymer and biotin via redox-responsive disulphide (-SS-) linkage, and DOX was subsequently encapsulated. The developed nanoparticles show redox-responsive behaviour in redox-responsive environment of tumour cells and release the co-drugs, thus enhancing the therapeutic efficacy of anti-cancer drugs.

Song et al. (2016) prepared reduction-responsive prodrug NPs for the self-delivery of DOX in breast cancer. In this system, two molecules of DOX were linked through disulphide linkage which formed self-assembled nanoparticles. Release study indicates that DOX NPs exhibited redox-responsive activity and enhanced cellular uptake level substantially compared with free drug DOX.

A mixed micelles system for the combination delivery of DOX and PTX in cancer therapy was prepared by Zhao et al. (2017). The drug release study indicates that the micelles released the maximum drug in reducing environment of GHS which demonstrated its redox-sensitive nature and also shows the significant cytotoxicity towards the lung cancer cells (A549 and B16).

3.3 Enzyme-Responsive Drug Delivery Systems

Just like pH, the distribution of enzymes in human tissues is non-homogeneous. Also, each tissue is composed of different types of enzymes having different expressions. For cancer cells, the expression level for these enzymes is higher than that of the normal cells (Badeau and DeForest 2019). Since enzymes are spatially as well as functionally specific, they have widespread applications in tissue engineering and drug delivery regimes. Recent years have witnessed the development of various enzyme-responsive drug delivery systems based on enzymatically cleavable chemical bond or linkage (Zhou et al. 2018). The most common enzymes that are used to develop enzyme-sensitive drug delivery systems for cancer drug delivery applications were shown in Table 6.1 (de la Rica et al. 2012). The major class of enzymes responsible for the cleavage of chemical bonds by hydrolysis are known as hydrolases. Proteases are the subclass of hydrolases which have an important role in cancer progression and possess the ability to degrade extracellular matrices and proteins.

The literature so far describes the development of various prodrug systems which are based on the protease activity. Esterases secreted in the endolysosomes of cells have been used in cancer therapy to release the drug. Hyaluronidase-1 is a glycosidic hydrolase which is secreted by the tumour cells and hence is found in the interstitial spaces of the tumour tissues. The hyaluronidase-1 mainly degrades the hyaluronic acid, therefore a large number of hyaluronic acid-based nano-drug delivery systems have been developed for the selective delivery of bio-agent. Histone deacetylase is an enzyme belonging to the class deacetylase, and it is responsible for cancer initiation and progression by regulating the expression and activity of cancer-causing proteins.

Table 6.1 Example of enzymes commonly used in targeted drug delivery

Class	Subclass	Enzyme
Hydrolases	Proteases	Cathepsin B Caps Caspase 1 thrombin collagenase chymotrypsin PSA
	Lipases	PLA_2
	Glycosidases	α-Amylase
	Others	Urease
Oxidoreductase		Glucose oxidase

4 Exogenous Stimuli-Responsive Drug Delivery System

4.1 Thermo-responsive Drug Delivery System

Generally the temperature of human body is close to 37 °C, whereas the temperature of the inflamed pathological sites or intra-tumoural environment is at a higher temperature of 40–44 °C (Alsuraifi et al. 2018). This temperature gradient is used as stimuli in the development of thermo-responsive drug delivery system. The temperature-sensitive polymers that respond in temperature change are the most common materials for the development of thermo-responsive drug delivery system.

Thermo-responsive polymers generally showed the phase transition at a specific temperature known as upper critical solution temperature (UCST) and lower critical solution temperature (LCST). These polymers are soluble below LCST whereas insoluble heating upon (LCST) (Tebaldi et al. 2018). The PNIPAAm and its derivative are most widely used in the preparation of thermo-responsive system because its LCST is about 32 °C which is close to temperature of human body. The LCST of PNIPAAm can be easily altered by random copolymerization involving different monomers leading to improved targeting and drug release (Raza et al. 2019a). The drug can be loaded at LCST and released above the temperature higher than LCST. Over the past few years, various thermo-responsive polymers or copolymers have been used in the development of thermo-responsive nanocarrier in cancer therapy (Kim and Matsunaga 2017; Wang et al. 2011).

Zhang et al. (2014) developed thermo-responsive polymer-encapsulated gold nanorods for the combined photo thermal- and thermo-responsive delivery of doxorubicin in lung cancer treatment where polymer shell of thermo-responsive polymer helped to prolong the circulation time and protect the drug from other body enzymes. Further the accumulation of drug was enhanced in tumour by NIR laser irradiation.

Recently, Tian et al. (2019) prepared thermo-responsive self-assembled nanoparticles based on 2-hydroxy-3-isopropoxypropyl hydroxyethyl celluloses (HIPECs) for the delivery of amphotericin B. The LCST of HIPEC was elevated from 21.1 to 56.1 °C, and the change in their size with temperature was observed by DLS and SEM technique. The drug delivery studies suggested a much faster release rate at temperatures above LCST where the majority of drug got released from the nanoparticles within 40 h.

4.2 Photo-/Light-Responsive Drug Delivery System

Among the various external stimuli, light has received considerable interest in drug delivery applications due to its non-invasive nature (Raza, Hayat et al. 2019). To stimulate the triggered drug release, light of infrared (IR) wavelengths can be used. The photosensitive biomaterials generally incorporate into the photoscissile moieties which are cleaved when exposed to specific wavelengths of light.

Generally, ultraviolet (10–400 nm), visible or near infrared (650–900 nm) was used to trigger the drug or other photosensitive materials. The near infrared (NIR) light is more suitable than UV light due to its deep tissue penetration without significant damage of body tissue, whereas UV cannot penetrate the deep tissue. A large number of light-responsive drug delivery systems were developed to achieve on-demand drug delivery in cancer therapy (Alsuraifi et al. 2018; Annis et al. 2009).

4.3 Magnetic-Responsive Drug Delivery System

Similar to other external stimuli, magnetic field is considered as another lucrative method to initiate triggered drug release (Price et al. 2018). Engineering the magnetically responsive materials in a specific manner may lead to the guided carrier accumulation and dispersion for payload delivery. In addition to delivery of chemo-drugs, the magnetic-responsive nanoparticles were also utilized to deliver the genetic components. The unique ability of magnetic-responsive nanoparticles is generating heat under the influence of external magnetic field. Under oscillating magnetic field, magnetic nanocarriers produce heat which cause significant changes in the structures of nanocarriers which release the payload (Hossen et al. 2019; Zhou et al. 2018).

4.4 Ultrasound-Responsive Drug Delivery System

In recent year, ultrasound technique is commonly used in clinical applications. The ultrasound technique is commonly used in clinical as diagnostic imaging technique (Zhou et al. 2018). Generally, 0.1–50 MHz frequency range of ultrasound waves is used in biomedical applications. Diagnosis is performed at low frequency, whereas treatment is occurring at high frequency. In addition to release of the drug, the US can also increase the permeability of carrier towards the cell barriers and blood-brain barrier by enhancing the temperature which increased the diffusion of drug. Ultrasound technique works on a specific process known as “sonoporation”. Recent study significantly indicates that the ultrasound-guided drug delivery can be used to cure different types of cancers that are anatomically accessible like liver cancer (Badeau and DeForest 2019).

5 Dual- and Multi-responsive Drug Delivery System

Due to the very complex nature of the cancer disease, one therapeutic approach is not sufficient to fully cure the cancer; therefore the combination of approaches was applied for better therapeutic effect. Various dual- or multi-responsive drug delivery

systems have been developed by combining two or more stimuli such as pH and redox, pH and temperature, temperature and magnetic field, enzyme and temperature and more other combination. So far various dual and multi-responsive drug delivery systems have been developed like Zhou et al. (2014) (Wang et al. 2016; Li et al. 2016; Hou et al. 2016), developed stimuli-responsive nanoparticles for the combinational chemo-phototherapy. The photosensitizer and chemotherapeutic agent were co-loaded on graphene oxide nanoparticles. The obtained results demonstrated that the combination therapy has great impact in cancer therapy as compared to individual therapy.

Similarly, Hou et al. (2016) prepared pH-sensitive prodrug nanoparticle for the targeted chemo-photodynamic therapy, where DOX was attached to polymer (PEG) through pH-sensitive (Schiff base) bond and subsequently photosensitizer Ce6 was encapsulated to form the combined platform. The nanoparticles showed the pH-responsive release behaviour and simultaneously release both the drug and photosensitizer at the acidic pH. Compared with free drug (DOX) and photosensitizer (Ce6), the nanoparticles exhibited greater antitumour efficacy against the cells, which was further observed by in vivo study. Thus the developed nanoparticle represents the combined effect of chemo- and phototherapy in cancer therapy.

Hou et al. (2016) developed charge-conversional nanoparticles based on histidine- and lipoic acid-grafted chitosan nanoparticles for improving the delivery of doxorubicin (DOX) in breast cancer therapy. Due to the negative charge of the histidine at physiological pH, the nanoparticle showed the stability during circulation, whereas charge conversion of histidine from negative to positive at acidic pH enhanced the cellular uptake activity. The nanoparticles exhibited rapid drug release in presence of acidic environment of cancer. In addition, the rate of drug release was also found higher in high concentration of reducing glutathione (GSH).

Similarly Xu et al. (2018) prepared pH- and redox-responsive nanoparticles, where DOX was conjugated with side amino groups of the corresponding poly(ethylene glycol)-*b*-poly(L-lysine) (PEG-*b*-PLL) polymer through 3,3′-dithiodipropionic acid. The triptolide was loaded into the inner core of polymeric micelles. The nanoparticles show negative charge at physiological pH, whereas it shows positive charge in the extracellular pH of tumour tissue. Most of the drug was released from the micelles in reductive environment of glutathione (GSH).

Yang et al. (2018) prepared dual-responsive charge-conversional NPs based on poly-L-lysine-lipoic acid (PLL-LA) for the effective delivery of doxorubicin (DOX). The NPs show negative charge at physiological pH, whereas it shows positive charge in the extracellular pH of tumour tissue. The prepared micelles released higher amount of drug in the reductive environment of GSH. The cell cytotoxicity clearly demonstrated the improved cytotoxicity of micelles against A549 cells which indicate the important role of dual responsiveness in cancer therapy. Recently, Ding et al. (2017) fabricated a multifunctional theranostic nanoparticle system for dual imaging and magnetic targeting, where Fe3O4 was loading chlorine-conjugated dextran nanoparticles. The prepared nanoparticle (DSSCe6@Fe3O4 NPs) exhibited dual near-infrared as well as magnetic resonance behaviour. The photosensitizer Ce6 shows their signal in the reductive intracellular environment, and the cellular

uptake of NPs was enhanced significantly in presence of magnetic field which demonstrated the important role of photodynamic therapy. Further the in vivo study demonstrated the effectiveness of DSSCe6@Fe3O4 NPs in cancer therapy.

6 Conclusion and Future Prospects

This chapter highlighted the various stimuli used to develop a large number of stimuli-responsive nano-drug delivery systems to improve the therapeutic efficacy and effectively deliver chemo-drugs clinically. The design of stimuli-responsive drug delivery system presents tremendous opportunities in drug delivery, biosensing and regenerative medicine. In addition, various combination strategies used for further enhanced the therapeutic efficacy have also been discussed in details. Currently, numerous preclinical studies based on stimuli-responsive systems have been published which shows their potential cancer therapy. Hope such type of smart systems will be beneficial with the combination of other therapies used in cancer therapy.

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Chapter 7
Plant-Mediated Synthesis of Silver and Gold Nanoparticles for Antibacterial and Anticancer Applications

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Contents

1 Introduction: Integration of Biology and Nanotechnology

Nanotechnology is one of the rapidly growing interdisciplinary areas of science that merges physics, chemistry, material science, engineering, biology, medicine, and allied branches of science. Nanotechnology manages materials that hold at least one measurement in nanometer scale. In recent times, engineered nanomaterials are broadly utilized in everyday regular products such as toothpaste, soaps, shampoos, cosmetics, and medicines (Song et al. 2009). Noble metal nanoparticles (NPs) carry an advantage of their surface plasmon resonance (SPR), plasmon light scattering, surface-enhanced Raman scattering (SERS), and surface-enhanced Rayleigh scattering optical properties over bulk mass materials and largely rely on their size, distribution, morphology, dielectric environment, and aggregation substance (Song et al. 2009; Jain et al. 2007; Nalwa 2000; Terenteva et al. 2015). These elite traits

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can be put to good use for controllable size dispersity, stability, strong adsorbing capacity, and biocompatibility to obtain engineered NPs (Lee et al. 2010). The embedded property of SPR enables silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) to own the exclusive optical behavior that let them to display intense color and corresponding specific extinction bands in their UV-visible (UV-vis) spectra of 420–450 and 530–540 nm wavelength, respectively (Rathi Sre et al. 2015; Ramteke et al. 2013; Sathishkumar et al. 2016; Dauthal and Mukhopadhyay 2013). AgNPs and AuNPs are widely utilized in different sectors including electronics, optical devices (Kamat 2002), textiles, cosmetics, energy, environment, catalysis (Dauthal and Mukhopadhyay 2012; Kim et al. 2003) determination of organic compounds in analytical chemistry (Laliwala et al. 2014; Song et al. 2014; Leesutthiphonchai et al. 2011; Miao et al. 2013), medicine, biological labeling (Nicewarner-Pena et al. 2001), tissue/tumor imaging, cancer and antimicrobial agent (Rathi Sre et al. 2015; Sathishkumar et al. 2016; Bindhu and Umadevi 2013; Parveen and Rao 2015; Huang et al. 2007; Nagajyothi and Lee 2011), drug delivery system (Mann and Ozin 1996), and sensor technology (Han et al. 2001). Besides AgNPs' and AuNPs' wide applicability, their synthesis has been emerged as a significant facet of research nowadays for targeted shape, size, chemical moieties, dispersity, and functions. However, toxicity carryover with the NPs synthesis that involves hazardous chemicals has always been a matter of question. An effort to combat toxicity, botanical herbs came forward with interesting therapeutic properties are encouraged among scientists. Readily available and simple to handle medicinal plants offer environmentally benign solutions over organic solvents. Therefore, herein present chapter emphasizes on medicinal plant-derived synthesis of silver and gold nanoparticles (p-AgNPs and p-AuNPs), their antibacterial properties, and cytotoxicity potential on cancer cell lines.

2 Approaches of Synthesis

In general, there are two approaches to obtain nanoformulations, namely, "top-down" approach and "bottom-up" approach (Fig. 7.1). Top-down approach involves size reduction of a suitable material to fabricate NPs (Meyers et al. 2006). Many types of physical and chemical procedures have been cited in literatures that are being applied for size reduction to attain nanoscale structures. These processes include mechanical grinding, thermal decomposition, pyrolysis, lithography, and laser ablation. Tube furnaces employed in top-down approach are operated through evaporation-condensation process at atmospheric pressure. These processes expend a great amount of energy for raising the furnace temperature. The base material is vaporized into a carrier gas inside the tube furnace. Additionally, these setups occupy large spaces and consume plenty of time to attain the operational thermal stability during the procedures. Such processes introduce structural imperfections in the faces of the surface of the product that turn to be a major drawback in the impli-

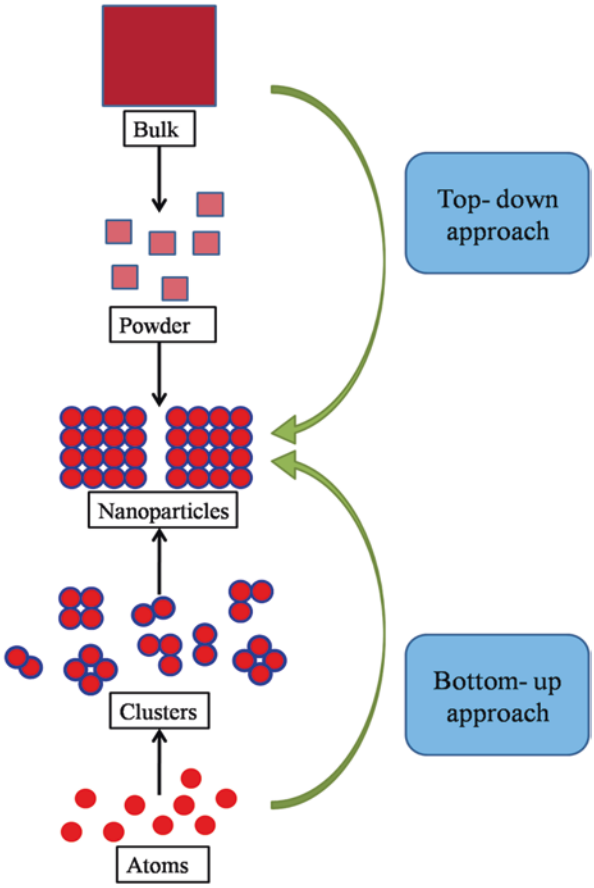

Fig. 7.1 General representation of top-down and bottom-up approaches for obtaining nanoformulations

cation of top-down approach. Surface structure is viewed as a fundamental and crucial property of NPs that is in charge for its surface science and other physical properties (Thakkar et al. 2010). On the other hand, in bottom-up approach or "self-assembly" approach, initially the atoms undergo nucleation process to form clusters that grow in a particular pattern to form NPs as a final product. Large amount of NPs can be fabricated in a short timeframe using bottom-up approach. It additionally diminishes the surface structure defects and sustains homogeneity of the product. The approach is mainly comprised of chemical and biological routes. The chemical route is the most regularly exercised procedure to obtain featured NPs. It can be implemented through a number of techniques including chemical treatment, sono-chemical application, electrochemical processes, polyol reduction, sol-gel processes, and vapor deposition. Chemical route utilizes compounds for reducing,

stabilizing, and surface capping agents such as sodium borohydride, sodium citrate, hydrazine, ascorbic acid, dopamine, levodopa, uric acid, ferulic acid, and polyphenols (Song et al. 2009; Guzman et al. 2009; Nezhad et al. 2010; Hormozi-Nezhad et al. 2017; Amjadi and Rahimpour 2012; Wang et al. 2007; Ozyurek et al. 2012). These chemicals compounds used during synthesis procedures are expensive as well as toxic and hazardous for living beings and environment. The by-products produced in synthesis reactions limit its applicability in clinical and medical field because of the associated biological risks.

However, biological route involves biological systems for the fabrication of nanomaterials which offers absolutely nontoxic, environment-friendly, and biofunctional alternatives to the chemical synthesis. Biological route for synthesis of NPs appreciates sustainability initiatives through clean chemistry (Fig. 7.2). Thus, biological route comes forward as a conjunction of nanotechnology and biotechnology that has gained significant attention in current scenario. NP syntheses using bacterial and fungal cultures as well as plants and their parts have stated a tremendous success due to their benign and safe application in medicine.

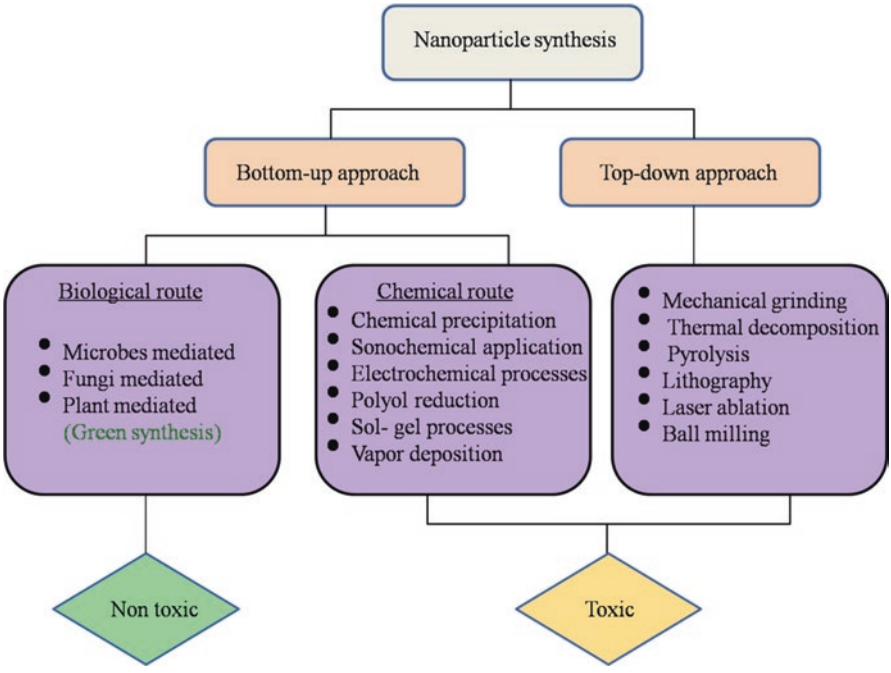

Fig. 7.2 A flowchart representation of biological and chemical routes of bottom-up approach

3 Plant-Derived Biosynthesis of Silver and Gold Nanoparticles

Plant derived silver and gold NPs recommend numerous benefits over bacteria and fungi derived NPs. Plants are easily accessible, non-infectious and hold economical merits together with a huge range of miscellaneous phytochemicals. A plethora of reports in literature have been documented stating whole plant, plant products, or plant parts such as leaf, stem, fruit, root, rhizome, tuber, flower, buds, and so forth mediated successful fabrication of functional p-AgNPs and p-AuNPs (Table 7.1).

Plants contain numerous primary and secondary metabolites and antioxidant compounds having therapeutic importance that are assumed to play premier role during synthesis of NPs. Botanic origin biomolecules including terpenoids, phenolic acids, flavonoids, flavones, tannins, and proteins have the ability to function as reducing and stabilizing agents to synthesize NPs (Sathishkumar et al. 2016; Dauthal and Mukhopadhyay 2012; Khan et al. 2016; Liu et al. 2018; Jain and Mehata 2017; Sheny et al. 2011; Philip and Unni 2011). A schematic diagram has been depicted in Fig. 7.3 representing participation of various phytochemicals present in synthesis and growth of NPs. These active components may either separately or synergistically function as reducing and stabilizing agents. A single phytoconstituent may also serve dual function of phytoreduction and stabilization. The biomolecules also help in preventing agglomeration by forming a bio-layer around the NPs. The choice of plants in other words phytochemicals is profoundly responsible for the determination of morphology, shape, size, dispersity, stability, and aggregation state of the formed NPs. During the synthesis process firstly, phytoconstituents bioreduce silver or gold cations to zero-valent state, followed by nucleation process to form clusters. Further these clusters continue to grow and biostabilized by the phytoconstituent-based capping agents to form p-AgNPs and p-AuNPs (Castro et al. 2011).

Scientist have developed different reaction parameters for the synthesis of plant-assisted NPs according to their objectivity of experiments including shape and size of NPs to be synthesized, functionality, application, and type of phytochemicals. A typical synthesis reaction is initiated by mixing plant extract into salt solution followed by incubation, centrifugation to harvest synthesized NPs, and purification processes (repetitive washing and drying). Here, one-pot plant-mediated synthesis signifies "green chemistry" that offers nontoxic, environment-friendly, cost-effective, biocompatible NPs. In general the procedure can be explained as a four-step process. In step 1, air-dried powdered or fresh plant material is extracted in water. Thereafter, in step 2, plant aqueous extract is added drop by drop in the flask containing precursor solutions (silver nitrate ($AgNO_3$) or chloroauric acid ($HAuCl_4$)) with continuous shaking in a ratio of 9:1. Further step 3 follows – continue stirring the reaction flask for 24 h under room temperature. The colorless $AgNO_3$ and $HAuCl_4$ solution turned to dark amber and pink-purple color solution, respectively. The color change is the indication of the synthesis of p-AgNPs and p-AuNPs. The prepared NP colloidal solutions are harvested in step 4 through centrifugation to

Table 7.1 Different medicinal plants utilized for synthesis of p-AgNPs and p-AuNPs of different morphology

NPs	Shape	Size (nm)	Plant name	Family	Habit	Part used	Bioactivity	Reference
Ag; Au	Spherical; spherical and cubic	33.67; 100	*Allium cepa*	Amaryllidaceae	Herb	Bulb	Nd, antibacterial	Parida et al. (2011); Saxena et al. (2010)
Ag, Au	Spherical, triangular	15.2–4.2, 350	*Aloe vera*	Asphodelaceae	Herb	Leaf	Nd	Chandran et al. (2006)
Ag	Spherical	30	*Arbutus unedo*	Ericaceae	Tree	Leaf	Nd	Kouvaris et al. (2012)
Ag, Au, Ag/Au; Ag	Flat, platelike; spherical	5–35; 41–60	*Azadirachta indica*	Meliaceae	Tree	Leaf	Nd; biolarvicidal	Shankar et al. (2004); Poopathi et al. (2015)
Au	Triangular, spherical, nanorods	200–160, 40–290	*Beta vulgaris*	Amaranthaceae	Herb	Pulp	Nd	Castro et al. (2010)
Ag	Irregular	20–35	*Calotropis gigantea*	Apocynaceae	Herb	Leaf	Larvicidal	Priya et al. (2014)
Ag; Au	Cubical; spherical	402; 10–22	*Calotropis procera*	Apocynaceae	Herb	Flower; latex	Nd; cytotoxicity	Babu and Prabu (2011); Das et al. (2011)
Ag, Au	Spherical	53.2, 31.7	*Camellia sinensis*	Theaceae	Shrub	Leaf	Nd	Jia et al. (2015)
Ag, Au	Spherical, spherical	20, 21	*Cassia auriculata*	Caesalpinioideae	Tree	Leaf	Anticancer	Parveen and Rao (2015)
Au	Rectangular and triangular	55.2–98.4	*Cassia fistula*	Fabaceae	Tree	Bark	Hypoglycemic	Daisy and Saipriya (2012)
Ag, Au	Quasi-spherical	12, 10	*Chenopodium album*	Amaranthaceae	Herb	Leaf	Nd	Dwivedi and Gopal (2011)
Ag	Spherical	37.71–71.99	*Chrysanthemum indicum*		Herb	Flower		Arokiyaraj et al. (2014)
Ag, Au	Triangular, spherical	55–80	*Cinnamomum camphora*	Lauraceae	Tree	Leaf	Nd	Huang et al. (2007)

NPs	Shape	Size (nm)	Plant name	Family	Habit	Part used	Bioactivity	Reference
Ag	Circular edge	>100	*Cinnamon zeylanicum*	Lauraceae	Tree	Bark	Antibacterial	Sathishkumar et al. (2009)
Ag	Multi-shaped	>100	*Citrus limon*	Rutaceae	Tree	Leaf	Antifungal	Vankar and Shukla (2012)
Ag	Spherical	425	*Citrus sinensis*	Rutaceae	Tree	Peel	Nd	Kahrilas et al. (2013)
Ag	Spherical	44	*Coleus aromaticus*	Lamiaceae	Herb	Leaf	Antibacterial	Vanaja and Annadurai (2013)
Au	Anisotropic	26 ± 11	*Couroupita guianensis*	Lecythidaceae	Tree	Fruit	Antioxidant	Sathishkumar et al. (2016)
Ag	Flakes	38	*Crossandra infundibuliformis*	Acanthaceae	Shrub	Leaf	Nd	Kaviya et al. (2012)
Ag	Spherical	15–20	*Datura stramonium*	Solanaceae	Herb	Leaf	Antibacterial	Gomathi et al. (2017)
Ag	Spherical	5–20	*Desmodium triflorum*	Fabaceae	Creeper	Leaf	Antimicrobial	Ahmad et al. (2011)
Ag	Spherical and flower-shaped		*Dioscorea batatas*	Dioscoreaceae	Perennial	Rhizome	Antimicrobial	Nagajyothi and Lee (2011)
Ag; Au	Spherical, triangular, hexagonal; anisotropic triangular, prism, spherical	75	*Dioscorea bulbifera*	Dioscoreaceae	Creeper	Tuber	Antibacterial; Nd	Ghosh et al. (2012); Ghosh et al. (2011)
Ag	Spherical	20–118	*Erythrina indica*	Fabaceae		Root	Antibacterial, cytotoxicity	Rathi Sre et al. (2015)
Ag	Cubic	412	*Eucalyptus hybrid*	Myrtaceae	Tree	Leaf	Nd	Dubey et al. (2009)
Ag	Spherical	28	*Eucalyptus oleosa*	Myrtaceae	Tree	Leaf	Nd	Pourmortazavi et al. (2017)

(continued)

Table 7.1 (continued)

NPs	Shape	Size (nm)	Plant name	Family	Habit	Part used	Bioactivity	Reference
Ag	Spherical; spherical	30–60, 263.11	*Euphorbia hirta*	Euphorbiaceae	Herb	Leaf, latex	Larvicidal and pupicidal, antibacterial	Priyadarshini et al. (2012); Patil et al. (2012)
Ag	Spherical	16	*Ficus benghalensis*	Moraceae	Tree	Leaf	Antibacterial	Saxena et al. (2012)
Ag	Spherical	9	*Hibiscus cannabinus*	Malvaceae	Shrub	Leaf	Antibacterial	Bindhu and Umadevi (2013)
Ag, Au	Anisotropic, quasi-spherical	21, 39	*Lawsonia inermis*	Lythraceae	Tree	Leaf	Nd	Kasthuri et al. (2009)
Ag	Spherical	34–48	*Melia azedarach*	Meliaceae	Tree	Leaf	Antibacterial	Mehmood et al. (2017)
Ag	Spherical	–	*Mentha piperita*	Lamiaceae	Herb	Leaf	Antibacterial	MubarakAli et al. (2011)
Au	Spherical	100	*Mirabilis jalapa*	Nyctaginaceae	Herb	Flower	Nd	Vankar and Bajpai (2010)
Ag	Spherical	23.7	*Musa paradisiaca*	Musaceae	Perennial	Peel	Antimicrobial	Ibrahim (2015)
Ag	Spherical	18	*Ocimum sanctum*	Lamiaceae	Shrub	Leaf	Antibacterial	Ramteke et al. (2013)
Ag	Spherical	11–37	*Pandanus odorifer*	Pandanaceae	Tree	Inflorescence male	Genotoxicity	Panda et al. (2011)
Ag	Spherical	16–40	*Pelargonium graveolens*	Geraniaceae	Herb	Leaf	Nd	Shankar et al. (2004)
Ag, Au	Spherical	32–53, 65–99	*Phyllanthus amarus*	Euphorbiaceae	Herb	Leaf	Nd	Annamalai et al. (2011)
Ag	Spherical	17.6–41	*Piper longum*	Piperaceae	Creeper	Leaf	Cytotoxic	Jacob et al. (2012)
Au	Triangular and spherical	5–20	*Punica granatum*	Lythraceae	Shrub	Fruit	Antibacterial	Lokina et al. (2014)
Ag	–	3.2–6	*Raphanus sativus*			Root	Antibacterial	Khan et al. (2015)

NPs	Shape	Size (nm)	Plant name	Family	Habit	Part used	Bioactivity	Reference
Ag	Spherical	30, 29, 92, 73; 93	*Syzygium cumini*	Myrtaceae	Tree	Seed; leaf, seed	Nd; antioxidant	Kumar et al. (2010); Banerjee and Narendhirakannan (2011)
Ag, Au	Triangular, spherical, hexagonal	10–40	*Tanacetum vulgare*	Compositae	Herb	Fruit	Nd	Dubey et al. (2010)
Au	Spherical	–	*Terminalia chebula*	Combretaceae	Tree	Fruit	Antimicrobial	Kumar et al. (2012)
Ag	Triangular	87–998, 3200–7600	*Trachyspermum ammi*, *Papaver somniferum*	Apiaceae, Papaveraceae	Herb, herb	Seeds	Nd	Vijayaraghavan et al. (2012)

Nd not determined or not detected

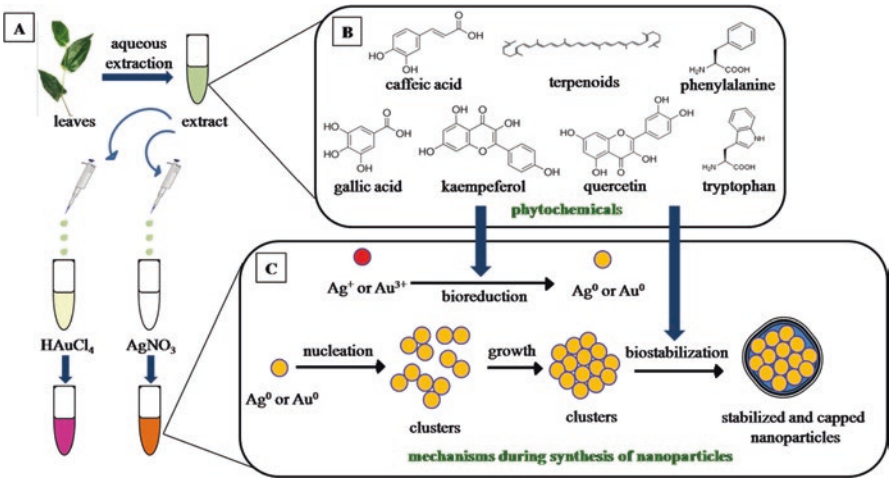

Fig. 7.3 Schematic diagram showing participation of various phytochemicals in synthesis and growth of NPs (**a**). Plant extract contains different types of secondary metabolites and antioxidant compounds that involved during synthesis as a bioreducing and biostabilizing agents (**b**); Ag^{+} or Au^{3+} undergo bioreduction in the presence of phytochemicals to convert into Ag^{0} or Au^{0}, following nucleation and growth processes to form cluster (**c**). These clusters get biostabilized by the phytochemical capping agents to form p-AgNPs or p-AuNPs

Fig. 7.4 A schematic diagram of plant-derived synthesis of p-AgNPs or p-AuNPs. Step 1, preparation of aqueous extract of plant material; step 2, drop-by-drop mixing of extract into the precursor solution in a certain ratio; step 3, color change of the reaction solution observed with continuous stirring; and step 4, centrifugation of colloidal solution to harvest the synthesized NPs

obtain synthesized NPs. Supernatant is discarded, and pellet settled near the bottom is washed with water. Synthesized NPs are air dried till constant weight and crushed to powder (Fig. 7.4). Thus, prepared NPs are weighed and stored in a glass tube in dark. Desired shape, size, and dispersity of NPs can be attained by managing the reaction parameters during the synthesis process. Concentration of metal salt solution and plant extract, volume ratio of metal salt solution to plant extract, incubation

time, temperature, and pH are regarded as effective parameters that can be altered during synthesis process for fabrication of diverse morphology of NPs. Some of the adopted parameters are listed in Table 7.2. Synthesized NPs are generally characterized for shape, size, surface area, and dispersity by combinations of techniques (Jiang et al. 2009).

4 Techniques for Characterization of Nanoparticles

Frequently used techniques are UV-vis spectrophotometry, scanning electron microscopy (SEM), transmission electron microscopy (TEM), powder X-ray diffraction (XRD), energy-dispersive X-ray spectroscopy (EDX), and Fourier transform infrared spectroscopy (FTIR). UV-vis spectrophotometry is the main applied technique that provides primarily evidence of NP formation. UV-vis spectrophotometry demonstrates characteristic absorption peak for surface plasmon resonance band in the wavelength range of 420–450 and 530–540 nm for AgNPs and AuNPs, respectively (Rathi Sre et al. 2015; Ramteke et al. 2013; Sathishkumar et al. 2016; Dauthal and Mukhopadhyay 2013). Observations for shape, size, and surface morphology of NPs are carried out under higher resolution in micrometer to nanometer range using SEM and TEM. EDX provides the qualitative and quantitative elemental composition of metal NPs (Strasser et al. 2010). Structural analysis is done through a predominant tool XRD that confers the information about both crystalline structure and phase of NPs (Sun et al. 2000). Surface chemistry of NPs is analyzed through FTIR technique. Functional groups and residues attached to the surface of NPs can be identified using FTIR.

5 Plant-Derived Silver and Gold Nanoparticles as Antibacterial Agents

Antibacterial activity of p-AgNPs and p-AuNPs has been accounted by several researchers in the literature. Ramteke et al. (2013) have reported antibacterial activity of 18 nm spherical shape p-AgNPs synthesized using leaf broth of medicinal herb *Ocimum sanctum*. The result revealed enhanced antibacterial activity of synthesized p-AgNPs against pathogenic strains *Staphylococcus aureus* and *E. coli.* Vanaja and Annadurai (2013) evaluated the antibacterial activity of *Coleus aromaticus* leaf-mediated 44 nm sized spherical p-AgNPs against *Bacillus subtilis* and *Klebsiella planticola* and suggested high toxicity of NPs (Vanaja and Annadurai 2013). In a study, spherical shape p-AgNPs of size range 9 nm were prepared using leaf of *Hibiscus cannabinus* that exhibited remarkable antibacterial activity against pathogens such as *E. coli*, *Proteus mirabilis*, and *Shigella flexneri* (Bindhu and Umadevi 2013). In another investigation carried out by Sathishkumar et al. (2009),

Table 7.2 Alteration in parameters adopted for medicinal plant-mediated nanoparticle synthesis by several researchers

Metal	Precursor	Conc. (mM)	Plant	Part used	Reaction mixture parameters				Characterization	λmax. (nm)	Bioactivity studied	Reference
					W:E	Temp. (°C)	IT (h)	pH				
Ag	$AgNO_3$	6	*Artocarpus heterophyllus*	Seeds	4:1	100	1	–	UV-vis, EDX, FTIR, SAED, SEM, TEM	410–420	Antibacterial	Jagtap and Bapat (2013)
Ag	$AgNO_3$	1	*Cassia auriculata*	Flower	9:1	100	5	–	UV-vis, SEM	450	Antioxidant	Velavan et al. (2012)
Ag	$AgNO_3$	1	*Cocos nucifera*	–	8:2	60	1	–	UV-vis, TEM, XRD	433	Larvicidal	Roopan et al. (2013)
Au	$HAuCl_4$	1	*Couroupita guianensis*	Fruit	19:1	70	1	7	UV-vis, DLS, EDS, FTIR, TEM, XRD	530	Antioxidant	Sathishkumar et al. (2016)
Ag	$AgNO_3$	1	*Cuminum cyminum*	Seeds	1:1	80	1	–	UV-vis, FTIR, XRD	530	Antibacterial	Jeyashree and Student (2017)
Ag	$AgNO_3$	1	*Erythrina indica*	Root	14:1	27	12	–	UV-vis, DLS, EDS, FTIR, TEM, XRD	438	Antibacterial, cytotoxicity	Rathi Sre et al. (2015)
Ag	$AgNO_3$	5	*Hibiscus cannabinus*	Leaf	149:1	100	–	–	UV-vis, EDX, FTIR, TEM, XRD	446	Antimicrobial	Bindhu and Umadevi (2013)
Ag	$AgNO_3$	1	*Mangifera indica*	Peel	9:1	80	0.25	11	UV-vis, FTIR, SEM, XRD	400–434	Antibacterial	Yang and Li (2013)
Ag	$AgNO_3$	1.75	*Musa paradisiaca*	Peel	49:1	40–100	72	4.5	UV-vis, FTIR, TEM, XRD	433	Antimicrobial	Ibrahim (2015)
Ag	$AgNO_3$	1	*Piper nigrum*	Fruit	49:1	100	2	12	UV-vis, SEM	420	Antioxidant	Mani et al. (2012)
Ag	$AgNO_3$	1	*Prunus armeniaca*	Fruit	9:1	60	8	4.8	UV-vis, DLS, FTIR, TEM, XRD	435	Antioxidant	Dauthal and Mukhopadhyay (2013)
Au	$HAuCl_4$	1	*Prunus armeniaca*	Fruit	1:1	60	8	2.9	UV-vis, DLS, FTIR, TEM, XRD	537	Antioxidant	Dauthal and Mukhopadhyay (2013)

Conc concentration, *W:E* water to extract volume ratio, *Temp* temperature, *IT* incubation time, *UV-vis* UV-visible spectrophotometry, *DLS* dynamic light scattering, *EDX* energy-dispersive X-ray spectroscopy, *FTIR* Fourier transform infrared spectroscopy, *SEM* scanning electron microscopy, *TEM* transmission electron microscopy, *XRD* X-ray diffraction

nano-sized silver particles have been prepared utilizing stem-bark extract and powder of *Cinnamon zeylanicum.* The synthesized p-AgNPs were assessed for antibacterial studies against *E. coli* BL-21 strain, and EC_{50} value was found $11 \pm 1.72\ mgL^{-1}$. In the study of Rathi Sre et al. (2015), root extract of *Erythrina indica* was employed in the synthesis of p-AgNPs. The obtained NPs were spherical and ranged between 20 and 118 nm possessing good antibacterial properties against E. coli, B. subtilis, Micrococcus luteus, Staphylococcus aureus, Salmonella paratyphi, and Salmonella typhi as compared to control. In another study reported by Khan et al. (2015), 3.2–6 nm p-AgNPs were synthesized from root extract of *Raphanus sativus.* The NP synthesis was done in the presence and absence of starch and cetyltrimethylammonium bromide, and antibacterial potential was determined against *S. aureus* and *E. coli*. They suggested that synthesized p-AgNPs revealed effective antibacterial agent as they were able to cause structural changes in protein cell wall. Saxena et al. (2012) demonstrated the antibacterial effects of *Ficus benghalensis* leaf extract-mediated p-AgNPs against *E. coli*. They proposed high surface to volume ratio of p-AgNPs that enabled better contact of p-AgNPs with bacteria causing antibacterial effects. Nagajyothi and Lee (2011) have synthesized p-AgNPs at 25 and 80 °C from rhizome of *Dioscorea batatas*. These synthesized p-AgNPs were evaluated against *B. subtilis*, *E.coli*, and *S. aureus.* Synthesized p-AgNPs exhibited antibacterial activity more effectively in gram-positive than gram-negative bacteria. p-AgNPs synthesized at 25 °C demonstrated more inhibitory activity on bacteria as compared to p-AgNPs synthesized at 80 °C. In another report, bactericidal activities of p-AgNPs and p-AuNPs against clinically isolated pathogens *E. coli* and *S. aureus* were studied by MubarakAli et al. (2011). *Mentha piperita* was used as bioreductant for both the NPs. Spherical shape 90 nm p-AgNPs and 150 nm p-AuNPs were observed to be effective against both the pathogenic isolates. A mixture of triangular and spherical shape p-AuNPs ranging between 5 and 20 nm in size were synthesized from fruit extract of *Punica granatum* (Lokina et al. 2014). The findings of the study revealed excellent antibacterial properties of p-AuNPs against human pathogens, namely, *S. aureus*, *S. typhi*, and *Vibrio cholerae*. Shamaila et al. (2016) synthesized p-AuNPs in 6–40 size range and explored its efficiency to lyse enteric bacteria *E. coli*, *S. aureus*, *B. subtilis*, and *Klebsiella pneumonia*. In the study of Das et al. (2013), *Sesbania grandiflora* leaf extract functioned as bioreductant to synthesize spherical shape p-AgNPs within the size range of 10–25 nm. These synthesized p-AgNPs were demonstrated to hold potent antibacterial effects against multidrug-resistant *Salmonella enterica* and *S. aureus*, human pathogenic bacteria. *Ocimum sanctum* leaf extract-derived fabrication of p-AgNPs and their antibacterial effects have been screened against both gram-negative and gram-positive microorganisms by Singhal et al. (2011). The study indicated that p-AgNPs possessing 4–30 nm size showed a better antibacterial action in a dose-dependent fashion than silver nitrate and standard antibiotic ciprofloxacin.

6 Antibacterial Modes of Action of p-AgNPs and p-AuNPs

The mechanisms behind the bactericidal potential of p-AgNPs and p-AuNPs have been partially learned in several studies, which suggested few modes of action explaining inhibitory effects of the NPs. Selectively inhibitory behavior of p-AgNPs toward gram-positive and gram-negative bacteria may assigned to the compositional differences of bacteria cell structure. Gram-positive bacterium cell wall comprised of a thickened layer of peptidoglycan backbone with linear polysaccharide chains containing peptide cross-linkages. Such arrangements provide rigidity to the cell and challenge penetration of foreign agents. However, gram-negative bacteria own thinner glycan layer with lipopolysaccharide membrane becoming susceptible to the antibacterial agents (Chaloupka et al. 2010; Kim et al. 2007; Shrivastava et al. 2007). Some researchers believe electrostatic forces play bactericidal role in p-AgNP-bacteria interaction. Positively charged p-AgNPs adhere with negatively charged bacterial cell and intervene the electrochemical gradient of bacterial plasma membrane leading to disturbed permeability (Fig. 7.5). In some cases, p-AgNPs may serve as a reservoir for Ag^+ in bio-nano-environment. Released Ag^+ attach with phosphorus of nucleic acids and sulfur of proteins and consequently intervene DNA replication and protein transcription leading to obstruction of cell division causing cell death (Fig. 7.5) (MubarakAli et al. 2011; Hatchett and White 1996; McDonnell and Russell 2001). The release of Ag+ from p-AgNPs may triggered the cell to undergo oxidative stress through generation of reactive oxygen species (ROS) and

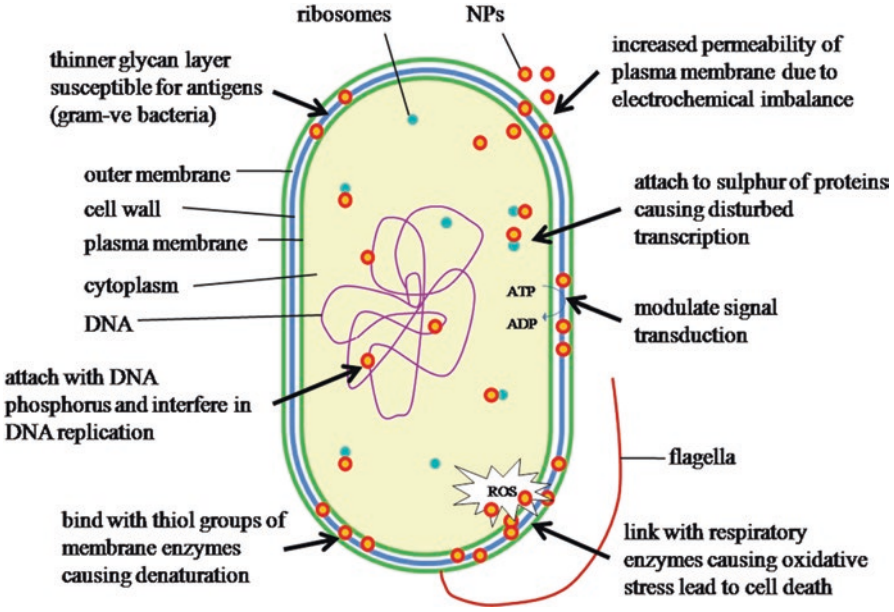

Fig. 7.5 Possible modes of action of p-AgNPs and p-AuNPs on bacterial cell

decreased levels of GSH leading to cytotixicity (Fig. 7.5) (Carlson et al. 2008). According to another explanation, released Ag^{+} may get linked to thiol groups of bio-enzymes and/or cell envelope proteins leading to denaturation, which ends up in cell death (Hajipour et al. 2012; Matsumura et al. 2003). According to reports of Shrivastava et al. (2007), p-AgNPs have been stated to modulate the signal transduction in gram-negative bacteria (Fig. 7.5). A majority of bacterial cell signal transductions are known to be regulated by protein substrate phosphorylation. p-AgNPs have been found to associate with dephosphorylation of peptide substrates on tyrosine residue which terminate the phosphotransfer signaling systems and ultimately lead to cell death (Shrivastava et al. 2007).

p-AuNPs have shown conflicting results as an antibacterial agent. Some scientists have shown p-AuNPs' antibacterial activities, whereas most of times, it has not proven so (Lokina et al. 2014; Parida et al. 2011; Castro et al. 2010; Kasthuri et al. 2009; Vankar and Bajpai 2010). These findings may infer that p-AuNPs mediated antibacterial activity is explored less, however, its role as nano-carriers in drug delivery systems possibly facilitate antibacterial activity (Brown et al. 2012; Ahangari et al. 2013). On the contrary, numerous scientific investigations have proven the antibacterial effects of p-AuNPs (MubarakAli et al. 2011; Naveena and Prakash 2013; Senthilkumar et al. 2017). The chemical constituents of plant parts or microorganism involved in the fabrication or bio-coatings of AuNPs were found to be associated with the surface modifications that may contribute toward antibacterial traits of NPs (Zhou et al. 2012). Bactericidal properties of NPs primarily depend on shape and size of the particles. In this context, NPs that are small and spherical in shape have better accessibility towards the larger surface area of the bacterial cell prompting lethal consequences (Saxena et al. 2012; Shrivastava et al. 2007).

7 Cytotoxic Studies of p-AgNPs and p-AuNPs

A plethora of investigations have been carried out in literature on cytotoxic activities of plant-derived green synthesized p-AgNPs and p-AuNPs against several models of cancer cells. In this context, some relevant works of the researcher have been concluded to present a brief investigation performed on cytotoxic effects of p-AgNPs and p-AuNPs on different cancer cells. Rathi Sre et al. in 2015 have demonstrated the biosynthesis of p-AgNPs from *Erythrina indica* and showed cytotoxic effects of NPs in breast and lung cancer cell lines in their study (Rathi Sre et al. 2015). The study concluded that 20–118 nm size p-AgNPs showed excellent cytotoxicity against MCF-7 and HEP G2 indicating its applicability as promising chemopreventive agent. In another recent study conducted by Lalitha in 2015, cytotoxic effects of *Alternanthera sessilis*-synthesized p-AgNPs has been studied (Lalitha 2015). The cytotoxic potential was estimated using MTT test against breast tumor model MCF-7 cell with considerable cytotoxic activity having IC_{50} value of 3.04 μg mL^{-1} in contrast to that of standard drug cisplatin. The authors suggested that the cytotoxicity was ascribed to spherical shape and smaller size (10–30 nm) measured through

TEM micrographs. The outcomes of the investigation revealed potent therapeutic efficacy of green p-AgNPs and their scope for future course anticancer drugs development in the field of medicine. The study of Jang et al. in 2016 revealed the selective cytotoxicity of synthesized p-AgNPs from aqueous extract of *Lonicera hypoglauca* flower that induced apoptosis to breast cancer MCF-7 cell lines, whereas there was no observed toxicity on RAW 264.7 normal immune cell lines. Recently, Maity et al. in 2018 proposed biogenic p-AgNPs as a considerable chemotherapeutic formulation in cancer therapy (Maity et al. 2018). Spherical shape p-AgNPs with a mean size of 2.33 nm have been synthesized from ethanolic extract of *Calotropis gigantea* latex and examined for its cytotoxic effects. Synthesized p-AgNPs showed in vitro cytotoxicity against Ehrlich's ascites carcinoma, Jurkat, and breast cancer MCF-7 cells at respective IC_{50} doses of 5.6 μg mL^{-1}, 11.99 μg mL^{-1}, and 13.33 μg mL^{-1}. Synthesized p-AgNPs showed no cytotoxic effect on mice and human lymphocytes. Further, the authors explored mechanistic actions of p-AgNP treated Ehrlich's ascites carcinoma cells. Here, NPs were found capable to induce significant chromatin condensation, DNA fragmentation, and arrest at G2/M phase of cell cycle progression along with upregulation of Bax:Bcl-2 ratio and caspase-3 proteins. Venugopal et al. (2017) synthesized p-AgNPs from *Piper nigrum* extract and demonstrated cytotoxicity potential against two different cancer cells, namely, MCF-7 (breast) and A549 (lung) cancer cells, in vitro (Venugopal et al. 2017). The biosynthesized p-AgNPs obtained were in the size range of 5–40 nm. Various concentrations between 10 and 100 μg of p-AgNPs have been evaluated for cytotoxicity, and the outcome indicated that the p-AgNPs were significantly effective against MCF-7 and Hep-2 cells when compared with *Piper nigrum* extract in a dose-dependent fashion. Cytotoxic study of p-AuNPs synthesized from *Punica granatum* fruit extract was performed by Lokina et al. (2014). Authors demonstrated that mixture of triangular as well as spherical shape p-AuNPs ranging from 5 to 20 nm has potential toxicity on cervical carcinoma HeLa cell at concentration between 7.8 and 1000 μg mL^{-1}. Priya and Iyer (2015) synthesized p-AuNPs from extracts of different plants including *Camellia sinensis* (green tea), *Coriandrum sativum*, *Mentha arvensis*, *Phyllanthus amarus*, *Artabotrys hexapetalus*, *Mimusops elengi*, *Syzygium aromaticum*, and *C. sinensis* (black tea) and investigated on MCF-7 cells for anticancer ability. The outcomes of the experiment suggested that p-AuNPs hold equivalent good cytotoxic effects as standard drugs tamoxifen and letrozole. Concentration as low as 2 μg mL^{-1} p-AuNPs was capable of inducing cytotoxicity in cancer cells. Increasing trend of cytotoxic effects of p-AuNPs on cancer cells was observed to be associated in a concentration-dependent manner. p-AgNPs have been documented for their association with the intracellular biological molecules such as phosphate components of DNA, proteins, and nitrogenous bases. The cellular uptake of p-AgNPs activates the sequence of events forming free ROS, which interfere with physiological and biochemical mechanisms of bio-organelles. Eventually cellular dysfunctions trigger the collapse of cell membrane integration, oxidative stress, or apoptosis that compel cell to death (Moaddab et al. 2011; Satyavani et al. 2011; Maity et al. 2018). The various exciting approaches have been described about the target specific actions of NPs in drug delivery systems. The anticancer drugs could

be embedded with functional NPs activated with moieties to its surface, and the whole drug-NP complex acts as nano-carriers in drug delivery systems that would be capable to act against particular receptor at specified target sites without causing any harm to other normal cells (Lalitha 2015; Zhang et al. 2015).

8 Conclusion

Plant synthesized p-AgNPs and p-AuNPs have largely attained the global interest for its multifaceted advantages. Intrinsic features including environment benign, economic and facile encapsulation, as well as biocompatibility of p-AgNPs and p-AuNPs makes them preferable over chemically synthesized NPs. p-AgNPs and p-AuNPs have shown to be accordingly engineered for targeted dimensions, morphology, and crystal structure via controlling the synthesis reaction parameters. Medicinal plants offer a wide variety of phytochemicals such as phenolic compounds, flavonoids, terpenoids, and proteins that hold antioxidant, antimicrobial, and anticancer properties. These active molecules of plant extracts were known to have a significant role in reduction and capping of NPs. The herbal moieties add their therapeutic properties to p-AgNPs and p-AuNPs during synthesis and improve NPs bioactivities. Scientists have reported very promising antibacterial and cytotoxic properties of p-AgNPs and p-AuNPs against numerous disease-causing bacteria and several cancer cell lines, respectively. Possible mechanism associated with these pharmacological actions of p-AgNPs and p-AuNPs has suggested their scope in target-specific drug delivery, conjugating ligands and clinical diagnosis of diseases.

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Chapter 8
Challenges in Nanobiosensor Aiming Bioscience Applications

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Contents

1 Introduction

Semiconductor oxide material-based pH sensors have been developed in numerous sensing fields such as biosensors, environment sensing, chemical measurements, and clinical investigations. However, the measurement of pH was considered as one of the most important requirements in biotechnology, analytical chemistry, and medicine applications. Bergveld fabricated the ion-sensitive field-effect transistor (ISFET) for pH sensor measurements in the 1970s (Bergveld 1970). Based on the ion-sensitive field-effect transistor, Yuqing et al. (2005) and Yin et al. (2000) developed a new structure of the pH measurements denoted as the extended gate field-effect transistor, which offers numerous advantages such as simpler packaging, low cost, and flexibility as compared to ion-sensitive field-effect transistor (Chou et al. 2005; Chou et al. 2008). Numerous studies have been carried out using metal oxides as pH-EGFET membranes. Guerra et al. (2009) fabricated V_2O_5 thin film as pH-EGFET sensor by using sol-gel technique. They explored the interaction between the membranes with the solution charges and calculated the sensor sensitivity. The

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results indicated a linear behaviour towards the pH values in the saturation region. The sensitivity of the sensor could be achieved by using measurements between reference electrode voltage and drain current. It can be observed the dislocation towards higher applied voltages as a function of the pH values. The fabricated film was examined as a pH-EGFET sensor within the range of 2–10 with an estimated sensitivity of 58.1 mV/pH. The value of the pH sensitivity of the pH sensor indicated a good value and approach to theoretical value (59.2 mV/pH).

Guidelli et al. (2012) deposited V_2O_5/WO_3 mixed thin film on glassy carbon substrates by using sol-gel method to fabricate pH-EGFET sensor. V_2O_5/WO_3 thin film offered a good result due to its lamellar structure, which allows for intercalation of the organic and inorganic species in the buffer solution. This produces several electrical properties of the solution due to the presence of ionic species and mixed valence associated with the proliferation of protons in the aqueous phase. The results indicated that the increase in the volumes of the solutions for the fabrication of the film leads to a wide change in the current compared to bulk, which causes a low response to the sensor. The drain to source current as a function of time for varying pH values was achieved by using MOSFET system in the linear regime. A sample test of a pH value ranging from 2 to 12 indicated a small current drift over a period of the time while testing the sample for each pH value takes longer to distinguish between each solution. V_2O_5/WO_3 thin film was tested as membranes in pH-EGFET sensor with sensitivity of 68 mV/pH in the linear regime and saturated sensitivity of 1.36 $\mu A^{1/2}$ pH^{-1}. The results indicated the sensitivity of the pH sensor was higher than theoretical value.

Vieira et al. (2012) evaluated V_2O_5 nanostructures as pH sensing application. The V_2O_5 nanostructure was deposited using hydrothermal method. The sensitivity of V_2O_5 nanostructure was calculated based on the resulting voltages for 3 minutes. The results found indicated that the sensitivity of V_2O_5 nanostructures was approached with the specified value (59.2 mV/pH), and it is an excellent value as compared with other metal oxide membranes. The work revealed several advantages for the fabrication of low-cost pH sensor membrane with favourable applications in the biosensor devices. Vieira et al. (2016) fabricated V_2O_5 nanorods and polyallylamine hydrochloride as pH sensor on gold substrates by using spin-assisted assembly method. The sensitivity of polyallylamine hydrochloride (PAH)/V_2O_5 nanorod membrane was examined by immersing the grown nanocomposite in the different buffer solutions and measured the dependent of output voltage of the system along time. The sensitivity of PAH/V_2O_5 nanorod membrane was higher and close to the limit value as compared with the gold membrane without modifications. The results indicated the multi-layered nanocomposite was to be penetrable to the hydrogen ions diffusion and the V_2O_5 inner layers contribute to the sensitivity. PAH/V_2O_5 nanorod membrane exhibited sensitivity of 52 mV/pH which depends on the PAH/V_2O_5 nanorod layer numbers. The work revealed the possibility of the spin-assisted assembly method for the fabrication of pH sensor by combination of the PAH/V_2O_5 nanorods deposited on the gold substrates.

Guerra et al. (2009) fabricated hexadecylamine V_2O_5/HDA membrane as pH sensor on the glass carbon substrates using hydrothermal technique. The electrical

property of the V_2O_5/HDA membrane as pH sensor was studied by the current variation as a function of time. The results indicated the effect of the current interval in time. The current variation for the pH range 2–12 was 27 μA at 0 s. After 300 s, this current variation increased to 46 μA. Consequently, the sensitivity was calculated after V_2O_5/HDA membrane had been immersed in the buffer solutions for 300 s. Therefore, current variation to each pH solution can be attributed to the composition of different constituent ions. The sensitivity of V_2O_5/HDA membrane was 38.1 mV/pH for the different pH values at saturation region. Our experimental arrangement presented a reasonable response with deviation of 35%.

Guerra & Mulato (2014) deposited titanium oxide (TiO) nanorods by using hydrothermal technique. The sensitivity of the TiO NR membrane as pH sensor can be calculated by evaluating the association between gate-source voltage and drain-source current. It is observed a shift towards high voltages with increasing pH values of the solution. The pH sensor showed a linear relation and sensitivity of 49.6 mV/pH for the buffer solution in the range (2–12). A variation of 10% in drain current leads to only 1.5% variation in pH sensitivity for the range between 350 and 450 μA. The result revealed the average sensitivity was close to the limit value (59.2 mV/pH). Therefore, TiO nanorod was a promising material as pH sensor and can be used as a biosensor application. Lin et al. (2012) synthesized indium tin oxide (ITO) nanorod as pH sensor on the Si substrates using vapour-liquid-solid method. The sensitivity of the untreated ITO nanorod arrays was found to be significantly higher than the sensitivity of the film at 150 nm thickness. By using the photo-electrochemical treatment, the pH sensitivity of the passivated ITO nanorod arrays with 150 nm wavelength was improved to 57.21 mV/pH as compared to the unpassivated ITO nanorod structure. However, when the length of the nanorod increases, the pH sensitivity decreases due to the granular shape and the decrease in the sensitivity surface area. The results showed the ITO nanorod array can be a promising structure and a favourable method for enhancing the pH sensitivity.

Li et al. (2012) fabricated SnO_2 nanorods (NRs) as pH sensor using hydrothermal method. The SnO_2 NRs as pH sensor revealed the sensitivity and linearity were 55.18 mV/pH and 0.9952, respectively. On the other hand, the linearity and sensitivity of the thin film are 0.9930 8 and 48.04 mV/pH, respectively. Moreover, it exhibited better linearity of 0.9964 and a greater sensitivity 0.86 $mA^{1/2}$/pH in the saturation regime. These results were attributed to the increase of the surface ratio to the size of the nanorod structure to prepare more surface sites and large areas of sensitivity. The results indicated the hysteresis was 3.69 mV after the pH solution was changed as pH 7 → pH 3 → pH 7 → pH 11 → pH 7. Lee et al. (2011) synthesized ZnO nanorods and ZnO thin films as pH sensor by using vapour-cooling condensation system. The results indicated that the the pH sensitivity of the unpassivated and passivated ZnO nanorod membrane were 47.96 μA/pH and 2.58 μA/pH, respectively. It was found that the sensitivity improvement for nanorod passivation due to the decrease of the Fermi-level pinning influence which causes the surface states and dangling bonds. Abd-Alghafour et al. (2017) synthesized V_2O_5 nanorods (NRs) as extended gate field-effect transistor (EGFET) pH sensor for the first time using spray pyrolysis method. The results showed that the V_2O_5 NR pH sensor exhibits a

superior linearity of 0.9859 and high sensitivity of 54.9 mA/pH in the saturation regime. These results can be attributed to the ratio of particle size to the surface of the nanorods which increases the surface site number and oxygen vacancies, resulting in larger effective sensing areas. The synthesized V_2O_5 NRs as pH EGFET sensor can be used in the promising biosensor application.

2 pH Sensing Theory

The field of research related to pH sensing has attracted great attention in particular in biochemical and biological applications. The term pH is derived from a combination of p for the word power and H for the symbol of the element hydrogen (Yuqing et al. 2005). In the electrolytic solution, the following interaction equilibrium exists between the water (H_2O), the acid ion (H^+), and the alkali ion (OH^-):

$$H_2O \Leftrightarrow H^+ + OH^- \tag{8.1}$$

The pH of the solution represents the number of hydrogen ions (H^+), not the concentration of the solution itself. The definition in pH is expressed as:

$$\log 10\left(\left[H^+\right]\left[OH^-\right]\right) = pH + pOH = 14 \tag{8.2}$$

In chemistry, solutions with a pH of 7 are defined as neutral. As the amount of hydrogen ions increases, acidity increases, and solution becomes low in pH. On the other hand, as the amount of hydroxyl ions increases, concentration of hydrogen ions decreases due to the relationship given in Eq. (8.2). Therefore, solution becomes basic and has a pH value higher than 7. Low-pH electrolytic solutions are called strong acid solutions, while high-pH solutions are called strong bases. The extended gate field-effect transistor (EGFET) as pH sensor is another structure to generate FET isolation from the chemical solution, so a sensing membrane is deposited chemically at the end of the short signal line from the FET gate electrode (Yin et al. 2000). The pH-EGFET sensor is a device used to indicate the acidity and alkalinity of the electrolyte solution by measurement of dissolved hydrogen ion concentration. The interactions between the electrolyte ions and oxide surfaces can be explained by site binding model which characterizes the surface reactions and layer potentials at the oxide-electrolyte interface (Hsi and Langmuir 1985), as shown in Fig. 8.1. According to this model, the insulating surface includes hydroxyl groups which form binding sites. They negatively charged (by losing H^+) or positively charged (by acquiring H^+) depending on the hydrogen ion concentration in the electrolyte solution. The resulting surface charge depends on the number of charged site of one type more than the other and is a function of the pH solution. Therefore, hydrogen and hydroxyl ions are representing as potential determining ions of the

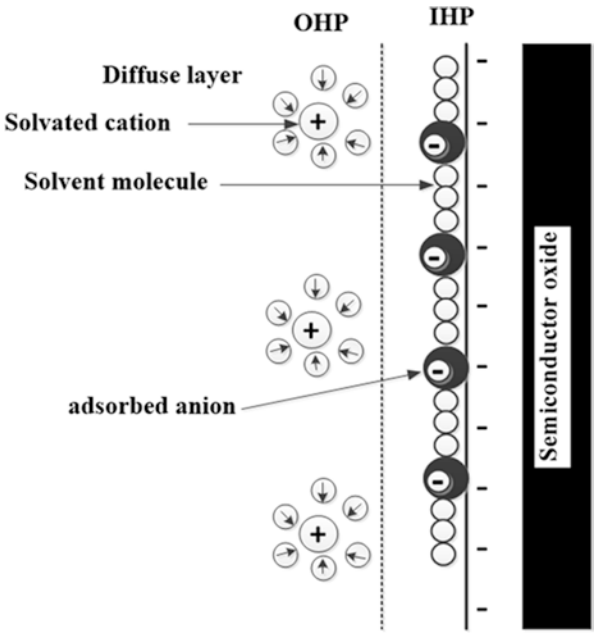

Fig. 8.1 Site binding theory of electrical double layer

surface. In addition, pH sensing theory electrolyte contains cations and anions called supporting electrolyte ions which form ion pairs with oppositely charged surface sites. This process can be indicated as surface complexation. The formation of surface complexes also readjusts the acid-base equilibrium and affects the surface charge.

The distribution of the electrolyte ions can be described by using Gouy-Chapman model (Bard et al. 1980). According to this model, electrical double layer is formed at the surface which contains a diffuse charge region and Stern inner layer. The diffuse charge region consists of the non-specifically absorbed ions which behave as an ionic cloud and are balanced by the uncompensated surface sites. Inner layer contains two planes, namely, inner Helmholtz plane (IHP) and outer Helmholtz plane (OHP). The first plane (IHP) is the position of the centres of the specifically adsorbed ions. The second plane (OHP) is the position of the centres of the hydrated ions with the closest approach to the solid. Therefore, the electrical double layer behaves as two capacitors diffused layer capacitance and Helmholtz capacitance. These two layers determine or even modulate the potential gradient across the solution, leading to varied currents. The higher the amount of H^+ ions in the solution, the thicker the charge diffusion layer, and the faster the charge diffusion layer is formed, leading to larger current values. The focus was on the surface/electrolyte interface (Yang 2012).

3 pH Sensitivity and Linearity

The MOSFET system (CD4007UB) was used to measure the IDS-VDS and IDS-VRFF curves for saturation and linear region, respectively. The value of the saturation current is calculated from the relation (Chi et al. 2000; Li et al. 2013):

$$I_{\mathrm{DS}} = \frac{\mu_n C_{\circ_x}}{2} \times \frac{W}{L}\left[\left(V_{\mathrm{REF}} - V_{\mathrm{T}}\right)^2 \left(1 + \lambda V_{\mathrm{DS}}\right)\right] \tag{8.3}$$

where μ_n is the electron mobility in the channel, λ is the channel-wavelength modulation factor, $C_{\circ_x}$ is the gate capacitance per unit area, V_{T} is threshold voltage related to the pH values, W / L is the ratio of channel width to length, V_{REF} is the reference electrode voltage, and V_{DS} is the drain source voltage. The square root of saturation current in Eq. (8.4) is given by (Chin et al. 2001):

$$\sqrt{I_{\mathrm{DS}}} = \sqrt{\frac{\mu_n C_{\circ_x}}{2} \times \frac{W}{L} \times \left(1 + \lambda V_{\mathrm{DS}}\right)} \times \left(V_{\mathrm{REF}} - V_{\mathrm{T}}\right) \tag{8.4}$$

Here V_{T} is the threshold voltage related to the pH value.

The sensitivity and linearity of the device can be expressed as:

$$\text{pH current sensitivity} = \frac{\Delta\sqrt{I_{\mathrm{DS}}}}{\Delta \mathrm{pH}} \tag{8.5}$$

The sensitivity and linearity of the pH voltage can be examined by using the equation as (Abdolkader et al. 2015):

$$\text{pH voltage sensitivity} = \frac{\Delta V_{\mathrm{REF}}}{\Delta \mathrm{pH}} \tag{8.6}$$

The hysteresis of the membrane can be defined as the chemical interaction between the H^+ and OH^- ions in the electrolyte solution and the surface defects of the membrane or slow reaction surface sites underneath the membrane surface (Yao et al. 2014). For hysteresis effect, the values of IDS-VG curves were calculated with different pH values when sensor membrane was immersed in different pH buffer solutions for 5 min. Then, the reference applied bias voltage for fixed drain current of 300 μA was extracted from each curve and plotted with different pH values.

4 Measurement Processes of pH Sensing

Figure 8.2 illustrates the pH sensing system setup; it consists of two Keithley 2400 source measurement units (SMUs) (Keithley Instruments, Inc., Cleveland, OH, USA). The units were connected to a personal computer (PC) via a GPIB-USB

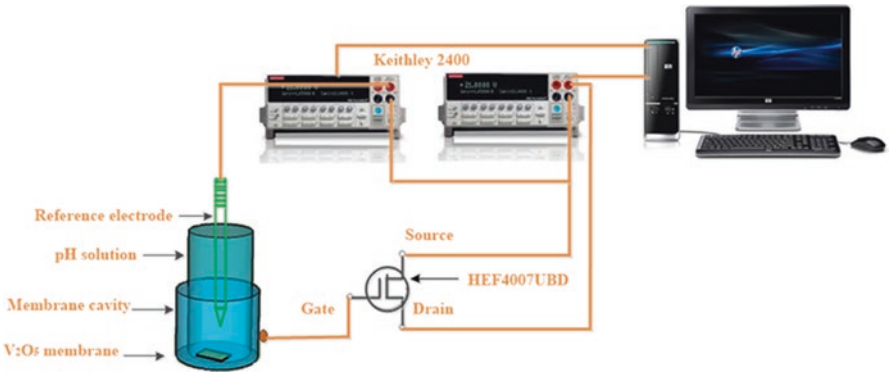

Fig. 8.2 Schematic diagram of EGFET as pH sensor

cable, and LabTracer software (Keithley Instruments, Inc., Cleveland, OH, USA) was utilized to start measurements and save data for further analysis. The commercial Ag/AgCl reference electrode was a standard reference electrode that gives constant potential during the entire measurement process. The standard reference electrode and sensing unit were directly immersed in the different pH solution (from Titrisol Products Company) and electrically connected with the gate of commercial standard MOSFET device (CD4007UB).

The reference electrode was placed in the same buffer solution and kept for 2 min at room temperature before measurement to provide a stable reference voltage for sensing element. The first process was used in the 2400 SMUs to apply the drain-source voltage (VDS) to the source and drain the terminals of the commercial standard MOSFET device (CD4007UB), while the second process was employed to apply the reference voltage (VRFF) to the reference electrode.

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Chapter 9
Topical Delivery of Drugs for Skin Disease Treatment: Prospects and Promises

Abinaya Mani and Gayathri Mahalingam

Contents

1 Introduction

In the last few years, the branch of technology and science is a rapidly emerging field of nanotechnology. The term "nanotechnology" was coined by Norio Taniguchi the professor of Tokyo Science University in 1974. The processes of semiconductor showed that the deposition of thin film might control through nanometers. The nanotechnology is the invitation of devices, structure, functional materials, and system with one superior feature. The scope of nanoscale particles are the rise and altering of the cell level between synthetic and biological materials (Du et al. 2007). It is also divided into three types (Singh et al. 2008):

- *Computational nanotechnology*: Handle with modeling and stimulating the multiplex nanometer-scale structure
- *Wet nanotechnology*: Handle with the biological system (cellular and enzyme components)

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- *Dry nanotechnology*: Handle with surface science, fabrication of silicon structure, inorganic material and carbon

Nowadays, the drug-resistant microbes are a starring role for the researcher community in the constant growth of active therapeutics. The nanoparticles synthesis showed the important application in biomedicine (cancer therapy, diagnostic and nutraceutical delivery, HIV and AIDS treatment, drug, and gene delivery), sensors, catalysts, electronic, optical fibers, bio-labelling, and agriculture (Salam et al. 2012; Austin et al. 2014; Moss 2013). The physicochemical properties of nanomaterial have prospective to develop a new system, structures, devices, and nanoplatforms with a wide variety of disciplines (Mirzaei and Darroudi 2017).

The nanoparticle formulation was typically implanted by conventional technique. This technique has a certain restriction like the generation of hazardous toxic chemicals and is also expensive. The researcher find the alternative perspective for nanoparticle synthesis amid through biological systems are safe, eco-friendly and also exploited as green principle process for synthesis. This biological-based method for nanoparticle formulation is generally classified into two processes such as:

1. Top-down process: The larger amount of nanoparticle are broken down into smaller particles (nanoscale) by different lithographic method, e.g., milling and grinding.
2. Bottom-up process: Self-assembly of atoms to new nuclei which grow into nanoscale particles (Fig. 9.1).

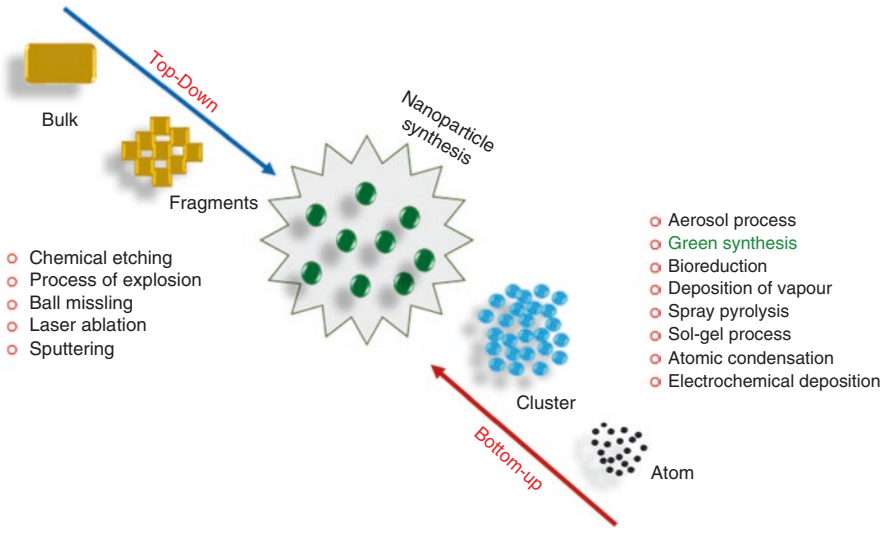

Fig. 9.1 Biological-based method for nanoparticle synthesis by "top-down" and "bottom-up" process

The alternative method for the physicochemical process of nanoparticle formation is biological nanoparticles. The present study highlights the green synthesis of nanoparticles are established through less cost, rapid, safe for human, and eco-friendly to use (Veerasamy et al. 2014). From the ancient time, the traditional medicinal plants are well exploited for different therapeutic compounds. The different medicinal plants which has been already explored into a wide range of applications in a different field such as agriculture, pharmaceutical, industrial, etc. The different nanoparticles synthesis using plant resources have a certain advantage like they are safe to handle and easily available and possess various biomolecules such as tannins, quinines, phenols, terpenoids, alkaloids, phenols, etc. These concepts of herbal synthesis of nanoparticles are termed as "herbonanoceuticals" (Gannimani et al. 2014; Das et al. 2014).

2 Plant-Based Green Synthesis of Nanoparticles

The word "green" nanoparticles don't mention the color yet the nanoparticle synthesis from metal salts using reduction properties of biologically active compounds. Green synthesis is an ecologically friendly material which was derived from bacteria, fungi, and plant sources. Designing of nanoparticles plays a main role for drug carriage system, to control the particle surface properties, size, and drug discharge through active sites to achieve a specific action of therapeutical process. This type of method isn't too expensive and harmful; the synthesis of nanoparticle was established using biological processes like bacteria, yeasts, molds, algae, actinobacteria, plant, and their byproduct (Fig. 9.2).

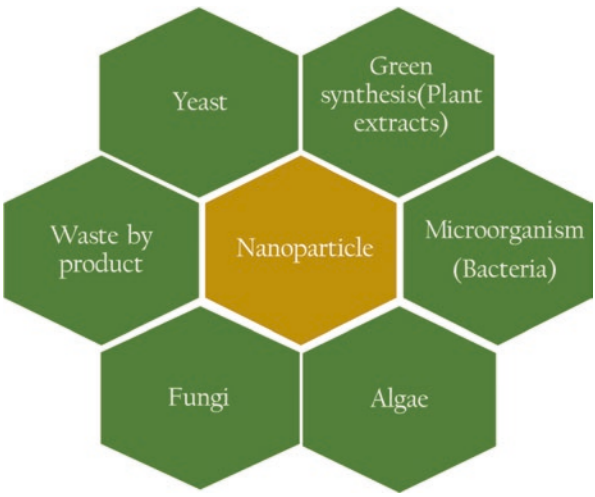

Fig. 9.2 Various synthesis of nanoparticles

Therefore, the synthesis of nanoparticle was carried out by reduction of plant molecules and microorganism such as enzymes, proteins, alkaloids, amines, and pigments (Shan et al. 2015; Nadaroglu et al. 2017). Among the various green synthesis techniques, the extraction from plant method is very effortless for metal nanoparticle synthesis, and also the nanoparticle formulation using bacteria and fungi is very simple at large-scale process. These sorts of products are mutually known as "biogenic nanoparticles."

During the *samhita* period (600–1000 BC), ayurvedic traditional medicinal plant sources was mostly used for numerous diseases system. At this particular period of time, the metals are used in powdered forms, known as "Ayaskrati." The vision of Ayurveda reveals that the combination of metal nanoparticles along with herbs formulation assists an effective therapeutic application. During this period, the metals were mostly used in the form of gold (Swarna), iron (Lauha), mercury (Parada), silver (Rajata), copper (Tamra), zinc (Yasada), lead (Naga), etc. The growth of *Rasashastra* was mainly owed by the presence of novel pharmaceutical methods for metal synthesis using ayurvedic research such as Jarana, Marana, and Shodana on seventh century AD (Kulkarni 2013). The active molecules present in herbs are able to reduce the nanoparticles and sustain them for the better therapeutic potential. Example, the plant extracts which consist of polyphenol act as reducing agent, and their −OH groups are involved in capping and sustaining the nanoparticles.

Traditionally, there are different methods for synthesis of metal nanoparticles such as gold, silver, copper, platinum, and palladium by using laser ablation, UV irradiation, photochemical reduction, lithography, aerosol techniques, and reduction of photochemical. Currently, the field of nanotechnology is stepped on into the synthesis of metal, gold, and silver particles by a natural organism. Mostly, due to the stability of particles, easy procedure, and possible application in biological imaging, antibacterial, gene sensing, chemical sensing, drug delivery, and gene mutation (Wei and Qian 2018). Mostly, a biological method for nanoparticle formulation has certain parameters like pressure, pH range, temperature, and different solvents (Doble and Kruthiventi 2007). The list on the table represents the current outing research work progress in the field of nanotechnology using green synthesis (Table 9.1).

3 Protein-Based Drug Delivery System

In existing era, drug delivery to a particular site is most challenging, because of therapeutic compounds that need to persist in a transport barrier throughout the body. Nowadays, nanoscale protein-based polymers gradually increase in the field of vaccine and drug delivery process. The release of drug which cross certain biological barriers through blood circulation and reached their molecular site of action (Hubbell and Chilkoti 2012). The polymer based biomaterial acts as a drug carrier system has a vital pharmaceutical application like degradation, mucoadhesive in

Table 9.1 Green synthesis of nanoparticles from different medicinal plants and their biological applications

Green synthesis	Nanoparticle	Size of the particles (nm)	Morphology	Application	Reference
Coleus forskohlii (root)	Silver	5–15	Spherical with small agglomeration	Wound healing process without scar or faint scar	Naraginiti et al. (2016)
	Gold	5–18	Nanorods, prisms, and triangles with no agglomeration	Antimicrobial, antioxidant, and anti-inflammatory properties	
Unused by-product of fruits: *Malpighia emarginata (pulp)* *Psidium guajava (peel, pulp, and seed)* *Passiflora edulis (seed)*	PLGA (poly(D,L-lactide-co-glycolide)	100–200	Spherical and monodisperse	Antimicrobial activity (*Listeria monocytogenes* and *E. coli*)	Silva et al. (2014)
Crocus sativus	Silver nitrate (AgNO3)	12–20	Spherical	Antimicrobial effect and biomedical application	Bagherzade et al. (2017)
Emblica officinalis (fruit)	Silver (AgNPs)	70	Crystalline nature with center cubic geometry	Antibacterial (Gram-positive and Gram-negative)	Ramesh et al. (2015)
Adansonia digitata (fruit and pulp)	Silver (AgNPs)	3–57	Spherical, polydispersed, and no accumulation	Antimicrobial and acts as eco-friendly agents	Kumar et al. (2016)
Sesuvium portulacastrum (callus and leaf)	Silver	5–20	Spherical	Antimicrobial	Nabikhan et al. (2010)
Azima tetracantha (leaves)	Gold (AuNPs)	80	Spherical	Human bacterial microbes (*Aeromonas liquefaciens*, *Enterococcus faecalis*, *Micrococcus luteus*, *Salmonella typhimurium*) Antifungal (*Candida albicans*, *Cryptococcus* sp., *Microsporum canis*, *Trichophyton rubrum*)	Qadr et al. (2016)
Azadirachta indica (leaves)	Gold	10–20	Spherical	*S. typhi* and *K. pneumoniae*	Grish (2018)
Ocimum gratissimum (leaves)	Silver	Average size 207	Stable, spherical, and polydispersed	Herbal gel formation – acne vulgaris Antibacterial (*Propionibacterium acne*, *S. aureus*, *E. coli*)	Prabhu et al. (2017)

(continued)

Table 9.1 (continued)

Green synthesis	Nanoparticle	Size of the particles (nm)	Morphology	Application	Reference
Vaccinium macrocarpon (cranberry fruit powder)	Silver	1.4–8.6	Spherical, polydispersed	Wound healing and antimicrobial (*S. aureus*)	Ashour et al. (2015)
Tinospora cordifolia and *Phyllanthus amarus*	Silver	33.7 35.4	Spherical, polydispersed	Antibacterial	Singh et al. (2014)
Selaginella myosurus (leaves)	Silver	Average 33.7–44.2	Crystalline aggregate, spherical with varied size	Anti-inflammatory, antimicrobial	Bell Ebanda Kedi et al. (2018)
Azadirachta indica (leaves)	Silver	15–35	–	Antimicrobial and antifungal and treatment for veterinary dermatology	Bansod et al. (2015)
Cannabis sativa (stem)	Fiber-AuNPs	12–20	Quasi-spherical	Inhibits anti-biofilm (*E. coli* and *P. aeruginosa*)	Singh et al. (2018)
	Core-AuNPs	12–20	Spherical, hexagonal, triangle, rod		
	Silver (core)	20–40	Spherical		
Ziziphus zizyphus (leaf)	Gold	3	Spherical, poly-crystalline, triangular and hexagonal platelets	Drug delivery without interfering human microbiota	Aljabali et al. (2018)
Galenia africana *Hypoxis hemerocallidea*	Gold	15–25	Spherical	Antibacterial activity against wound infection microbes (MRSA)	Elbagory et al. (2017)
Tiliacora triandra (leaf)	Gold	10–20	Spherical, polydispersed	Biocompatible and potential for nanocarrier material	Ndeh et al. (2017)
Eclipta prostrata (leaf)	Copper	23–57	Face-centered cubic	Therapeutic application, antioxidant	Min Chung et al. (2017)
Vitis vinifera (leaf)	Copper	–	–	Antibacterial (*S. aureus*, *E. coli*, *K. pneumoniae*, *B. subtilis*, and *S. typhi*)	Angrasan and Subbaiya (2014)
Cassia fistula (flower)	Copper	20 (μm)	Cluster and aggregate	–	Valli and Suganya (2015)
Moringa oleifera (leaf)	Silver	9–11	Crystalline	Antimicrobial (bacteria and fungi)	Moodley et al. (2018)

nature and drug discharge. The protein-based polymers was compared to the synthetic polymers based on their advantages likes:

- Natural resources
- Biodegradation
- Biocompatibility
- Water solubility
- Cost-effectiveness
- Environmental sustainability
- Stability
- Complex heterogeneity
- Nontoxicity
- Flexible route of administration

The outdated methods are inappropriate for chronic and insistent condition of drug delivery systems due to their duration, nontoxicity, and control of the dosage of drug delivery at a particular time. Animal proteins such as collagen, keratin, elastin, and silk are low-cost and sustainable. These types of protein have high biocompatibility and maintain the structural properties for biomedical application. Consequently, mechanical stable polymer materials encapsulated with drug act as a drug carriage system. Polymer-based material has various structures and shapes likes nanoparticle, micelles, gels, fibers, microsphere, scaffolds, hydrogels, and films. This type of polymer-drug delivery system can shield themselves from harsh gastrointestinal tract environment condition and also release the drug at an effective site without any side effects (Jao et al. 2017).

Biomolecules from plant sources are protein, carbohydrate, polysaccharide, and coenzymes with typical prospective to decrease the metal salt into nanoparticles and also show the tremendous controlling size of nanoparticles. The plant protein (Zein) was derived from maize, which are already shows that the drug delivery system has a progresses to interface of biological environment, retention time and absorption. The plant-based nanoparticles synthesized are gold, silver, and metal nanoparticles like other biosynthesis processes. Further plant-proteins are used for delivery of peptide, DNA, protein, and vaccines (Peng et al. 2016). The animal and plant protein, which have small peptides and hydrolysate, can castoff from animal (poultry and meat), aquatic (fish), and agriculture zones (Ferraro et al. 2016). The list on the table represents the protein-based polymer for drug delivery system (Table 9.2).

4 The Recent Development of Topical Delivery on Skin

The human skin acts as a shield against topical delivery (chemicals and microbes) and also reduces endogenous material. In the current era, the research of dermatological treatment is broadly focused on the novel treatment for skin diseases. This type of innovation might improve the dermal localization of active compound into the specific affected skin area through new nanoparticles which act as a drug carrier

Table 9.2 Protein-based synthetic and natural polymers act as binding agent for drug delivery system in therapeutic application

Nanoparticles	Drugs	Types	Application	References
Synthetic polymer				
Poly(isohexylcyano-acrylate)	Doxorubicin	Nanosphere hydrophobicity and biodegradation	Tissue distribution	Verdun et al. (1990)
Polyalkylcyanoacrylate	3H-dactinomycin and 3H-vinblastine	Degradability and sorptive properties	Antitumor	Couvreur et al. (1980)
Poly (lactide-co-glycolic acid)	SGC8 aptamer	Hydrophilic and hydrophobic	Cancer drug	Hung et al. (2014)
Silk elastin protein polymer nanoparticles	Drug trigger	Amphiphiles and micelles	Drug delivery	Xia et al. (2014)
Magnetic nanoparticles (Fe3O4 NPs)	Target drug delivery	Superparamagnetic	Anticancer and biomedical application	Yew et al. (2020)
PLGA – poly-L-lysine (PLL)-polyethylene (PEG) – transferrin nanoparticles	Daunorubicin	Spherical and uniform distribution of size	Antitumor efficacy	Bao et al. (2015)
Natural polymer				
Chitosan	Retinol Clobetasol-17-propionate	Hydrogel	Skin wrinkle, acne	Kim et al. (2006)
Lecithin-chitosan	Quercetin	Solution	Anti-inflammatory, antineoplastic	Senyigit et al. (2010)
Collagen-chitosan	*Aloe vera* gel	Gel (increase hydrophilicity)	Topical drug delivery	Tan et al. (2011)
Collagen	Different concentration of dextran and zinc oxide	Hydrogel/scaffolds	Anti-aging	Rajashree and Rose (2018)
			Antibacterial, wound dressing	Panea et al. (2016)
Polysaccharide (*G. birdiae*)	Silver nanoparticle (concentration)		Antibacterial and nano-medicine application	De Aragao et al. (2019)

material and release the drug directly to specific target cells. Later these nanomaterials enter via *stratum corneum* into the skin and build up at the particular target site (Gupta et al. 2012). To increase the transdermal delivery system, moreover, just modify physicochemical properties of drug or progress novel technology such as:

- Construct new molecules for carriage through various routes.
- Increase variability of poor candidates.

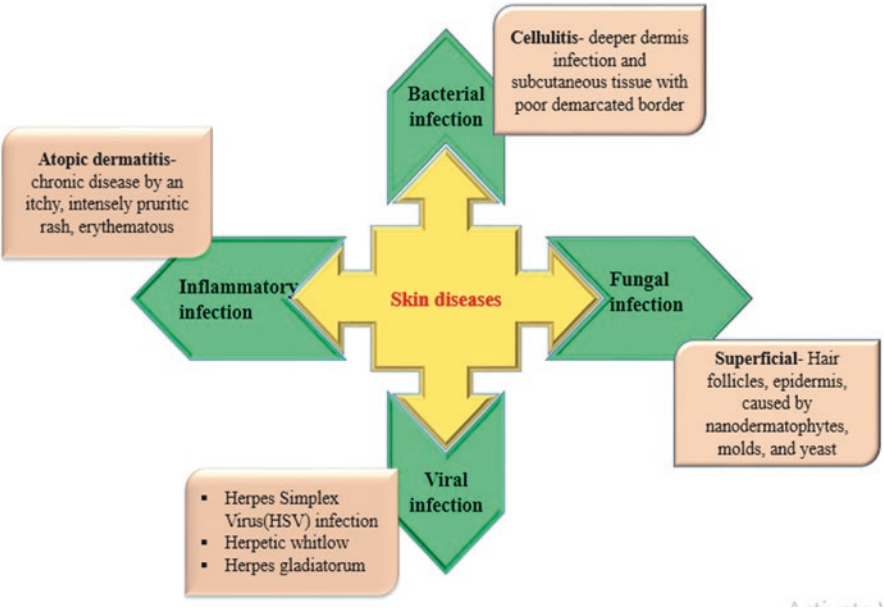

Fig. 9.3 Schematic representation of skin disorder which is significantly caused by infectious pathogen and inflammatory conditions

Topical delivery is always used in particular pathological sites of the skin where the drugs are entrapped by systemic absorption. These types of localization of drug are most significant for the treatment of dermatological process like psoriasis, eczema, skin cancer, and inflammatory, fungi, microbial, and viral infection that act as a main role for skin disorder. Among the different disorder, most significant and challenges skin diseases are certainly caused by infectious pathogen of skin flora and inflammatory conditions. The allergic contact dermatitis and atopic dermatitis of chronic inflammatory skin diseases was resulted in penetration of the inflammatory T cells with increased production of cytokines in lesions (Fig 9.3). According to the report of scientific research showed that the dermatologic diseases are precise severe and frequently effects on the population of developing countries (Sigmundsdottir 2010).

5 How Nanoparticles Used as Drug Delivery System? Nanoparticles Are Used as Drug Delivery System?

The nanoparticles have unique and significant characters such as the mass ratio of surface, which is greater than that of other material to allow catalytic reaction process, and also have their capacity to entrap and carry other compounds. The nanoparticles

act as a drug delivery system based on important criteria such as surface properties, control the particle size and release of pharmacologically active agents into the specific target site of action with dose-dependent manner. The characteristics of nanoparticles showed that the release of drug is feasible to diffuse into body tissue which has been unreachable before the target site (Mura et al. 2013), though there is no sign of particles if the nanoparticle matrix doesn't release the drug at a specific site. The drug release from nanomaterial is established on several features such as temperature, pH, and drug diffusion across nanoparticle matrix, drug solubility, adsorbed drug, a grouping of erosion, and diffusion processes (Son et al. 2017).

Most unique and possible nanocarrier used for drug delivery system is liposomes because of certain advantages like targeting on specific site of action, protecting the drug from degradation, and reducing toxicity or side effect. The disadvantage of the system is due to low encapsulation efficiency, less storage stability, and water-soluble drugs that are leaked due to the existence of blood components.

Another type of nanoparticle formation by polymeric nanoparticles is also known as nanosphere/nanocapsules that were established through their composition. This method is used to raise the stability of protein or drugs and also control the release of drug properties. Nanospheres are the homogenous system which is arranged in polymer chain reaction as parallel to that of surfactants in micelle formation. Nanocapsules are a heterogeneous system, where the drug is bounded to the polymer through reservoir composed (Vila et al. 2002).

Advantage of Nanoparticles as Drug Delivery System:

- Control release of drug and degradation process.
- No wastage of drug and enhance the bioavailability of drug at a specific target site for a sustained period time.
- Drug can be mixed with particles without any chemical response.
- Its plays a significant factor for preserving the drug.
- It improves the solubility of water-insoluble drugs.
- Progresses the prolong half-life of drug system circulation by reducing immunogenicity.
- It improves the therapeutic performance of drug over the conventional system without any side effects.
- It acts as drug delivery system based on the drug-carrying capacity, high specificity, and stability and controls and sustains release of the drug.
- Various types of drug administration and also have the capacity to transport hydrophilic and hydrophobic molecules.
- After reaching the target site, the drug is released from nanoparticle through diffusion, swelling, and degradation.
- Different types of nanoparticle such as polymeric, dendrimers, ceramic, and liposomes are mostly used for antimicrobial drug delivery system.

6 Application of Nanoparticles Used in Various Fields

Nanotechnology deals with various types of nanoscale material size which is lesser than 100 nm level (Laurent et al. 2010). Nanoparticle that was treated with particular compounds, atoms, and molecules for the formation of special properties such as materials and devices. These types of nanoparticles are used in various range of application like biomedical, food, environment, and industry (Fig. 9.4).

Natural nanoparticles are typically found in soils, plants, atmosphere, sediments, and natural water. In current decades, the applications of drug carriage system through nanoparticles are enriched in various divisions such as medical, pharmaceutical, and biological. The exploration of nanoparticle that arise the inhibition of protein and peptide aggregation process is related to some "misfolding diseases." This type of aggregation was transformed into amyloid fibrils process, which causes major neurodegenerative diseases such as Parkinson, Creutzfeldt-Jakob, Alzheimer, and etc. (Burke et al. 2013). The nano-biomedicines are prepared by nanoscale molecules which are mostly used in drug delivery system to progress the drug bioavailability. Through cell precision, the nanoscale molecules are target and discharge the drug at particular sites (Allen and Cullis 2004).

In vivo imaging is a tool of nanoparticles. The drug design system for therapeutic and pharmacological properties should be based on lipid and proteins based nanoparticles. For example, polymer-coated iron oxide nanoparticles interrupt the bacteria clusters and have been used for chronic bacterial infection treatment. The field of biomedical application of nanoparticles has greater benefits and valuable sources for human races. Nowadays, the novel approach of synthesize hybrid nanowires application for diagnosis and drug delivery system through optical devices (Hu et al. 2018). Another fascinating research work towards the plant

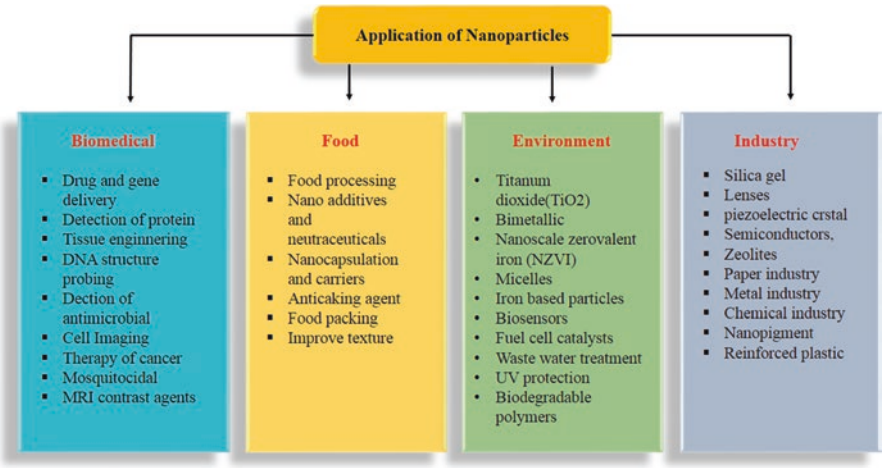

Fig. 9.4 The potential application of nanoparticles used in different fields

inspired-Ag lignin blended with nanoparticle-based adhesive hydrogel was established for dynamic catechol redox system. This type of hydrogel has an anti-infection property for repairing wound skin surface (Gan et al. 2019).

The National Nanotechnology Initiative which was held in the USA elucidated that the technology has a greater influence on nanoscale structure with unique properties for new applications. Nowadays, the field of nanotechnology is implant in food industry with highly impacts on each aspect of food organization from food production, cultivation, processing, packing, transportation, bioavailability of nutrients, and shelf life process (Doyle 2006; Cushen et al. 2012). The food technologist has greater challenges to progress food safety and supply foodstuffs. The food borne diseases were caused by meat, poultry, fruit, and vegetable products act as vehicles for the transmission of the human pathogens. The progression in the food industry is due to their unique and novel properties of nanoparticles that are commercially used. Nowadays, the food and food-associated products was mostly encompassed with nanomaterials, which may leads to be safety aspects in the view of public (Berger et al. 2010).

Nanotechnology has a larger potential of customer products and nanoscale materials are leads to improve the environmental condition in direct or indirect application. In direct technique the material was identify, inhibit and eliminate the pollutants and indirect method the nanomaterial was design to industrial cleaner processes for environmental responsible. Example: iron nanoparticles eliminated the contamination from ground water and soil. The nanoscale sensor progress the detection and tracking the contamination level in the soil (Mansoori 2003).

7 Conclusion and Future Prospects

In recent period, the upcoming promising fields is green nanotechnology, which has wide ranges of application in research, pharmaceutical, industry, environmental, food, health/cosmetic and biomedical. The future prospects of nanotechnology should generate and implantation of different novel materials, and devices which have a greater perspective in the field of biomaterials, electronic, medicine products, drug delivery at target sites.

It can also improve their stability to improving the drug loading, release, targeting, and interaction, biological barriers, and bio-destruction of active compounds. It should also focus on the problem created by novel technology arises from environmental, toxicity impact of nanomaterial and effect of global economics. The major problems like cytotoxicity of nanoparticles or degradation of materials and development in biocompatibility are the foremost concern for future research that should be stepped on.

The growth rate of topical drug delivery for the healthy annual range is 25%, oral drug delivery 2%, and inhalation delivery 20%. The list of topical drug delivery system will be increased in the future with novel emerging device and technology of nanoparticles. The effective delivery of drug will improve the quality of patient's

life which is connected with healthcare and recognition of pathogenic conditions, decrease the infectious diseases, and recover the clinical outcome of the patient. Nowadays, the field of nano-biotechnology should generate novel materials that are eco-friendly, cost-effective, and reliable.

Therefore, the synthesis of nanoparticles through the biological process is emerging as harmless and alternative to the conventional method. Therefore, the existing review reveals that the essence of green synthesis nanoparticle productions for topical drug delivery system was deliberately reported in various literatures so far. Undertaking the new technology and challenges can create this knowledge for years in research, development, and applications for future prospects.

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Chapter 10
Biosynthesis of Nanoparticles and Their Potential Application in Food and Agricultural Sector

Manasa Kumar Panda, Yengkhom Disco Singh, Rajendra Kumar Behera, and Nabin Kumar Dhal

Contents

1 Introduction

Nanoscience and nanotechnology is one of the most emerging areas of material science having various applications ranging from healthcare system to food (Chau et al. 2007) and agriculture sector. Nanoparticles have unique physical and chemical

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properties due to which these materials are used in various fields such as medicine, biotechnology, food science, agriculture sector, electronics, bioenergy and biofuel production, and other applications of materials science. Nanomaterials exhibit high aspect ratio than other compounds (Rajput 2015). Nanotechnology deals with the nanomaterial of size having 1–100 nm (He et al. 2019). Different encapsulation techniques can be used in entrapment of agrochemicals like fertilizer, pesticides, plant growth regulators, herbicides, and other active compounds by using formation of different means of polymers, dendrimers, and other nanoformulations. Encapsulation of nanoparticle with other materials allows plants to uptake and absorb the nutrients, and in return it helps in reducing the amount of waste that arises out of agrochemicals. Nanoformulation in agricultural drug delivery system improved the materials to have more solubility and stability in adverse environmental conditions. These nanoformulations provide firmly attachment of ingredients to the plant surface by preventing runoff into the environment. These promising materials are needed for continuous innovations and proper execution in encapsulation with other biological materials for the formulation of nanobiomaterials to mitigate the global warming and increasing food security (Zhang et al. 2019).

Agriculture is one such sector where the practice started since human inception and different technological innovations are included in time to time in order to produce sufficient and sustainable products for the mankind. The technology may include introduction of new hybrid verities, synthetic chemicals, biotechnology, and many more. However, human thrusts are unlimited, and by considering the advance technologies, researchers are now looking on exploitation of nanotechnology in improving agricultural practices (Fig. 10.1). Use of nanotechnology in food and agriculture sector will improve the quality and quantity of crops yield. Nanotechnology application has been an ongoing process for searching out several amicable solutions by taking environmental challenges for sustainable development and increased productivity. Nanomaterials in agriculture sector has certain goals: (1) reducing chemical treatment, (2) smart delivery of active ingredients, (3) minimizing nutrient loss, (4) more productivity, (5) and effective uses of drug against the plant pathogen. Plant breeding and plant genetic leads to enhancement of agricultural productions (Dennis et al. 2007). In plant genetic engineering, application of nanotechnology provides more effective system than traditional breeding technologies (Torney et al. 2007). Additionally, despite the fact that nanotechnology has great potential in the agriculture sector and its vast used are still in comparably marginal in the market as compared with other industrial sector.

Promoting of global food security is rendered by nanotechnology and its applications. The use of nanotechnology can provide bioavailability and nutritional value of the food (Srinivas et al. 2009). In fact, applications of nanomaterials in food science enhance the food security, extend shelf-life of post-harvest produce, improve flavour, maintain nutritional value, and can easily detect pathogen/toxins by using nano-biosensor.

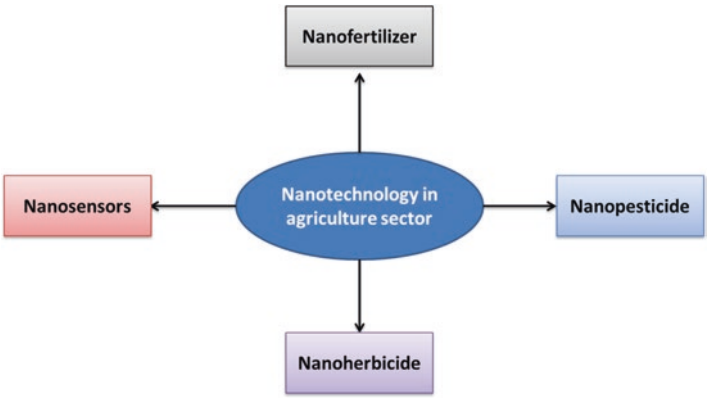

Fig. 10.1 Application of nanoparticles in agriculture sector. Four different aspects of nanomaterials are utilized in agriculture sector: nanosensor, nanopesticide, nanofertilizer, and nanoherbicide

This particular material is exhibiting a perfect character for rendering in food technology and agricultural science. Nanoparticle in processing and packaging of food materials is quite a success system (Weiss et al. 2006; Durán and Marcato 2013). Majority of the researchers in food and packaging emphasize on using nanoparticles to enhance post-harvest shelf-life. Food industries clearly benefitted from nanotechnology in particular food processing, packaging, distribution, and other functional foods (Fig. 10.2).

2 Biosynthesis of Nanoparticles

There are different techniques of synthesis of nanoparticles, and each technique will produce nanoparticles of different physical properties, size, and characters (Feng et al. 2010).

There are different methods of biosynthesis of nanoparticles. Broadly, the approach for such biosynthesis may be through chemical, physical, hybrid, and biological methods (Diallo et al. 2017; Liu et al. 2010; Chaudhry et al. 2018; Singh et al. 2019). Although physical and chemical methods of synthesis are quite popular and are more advantageous, the use of such chemicals and toxic materials causes environmental pollution and global warming which greatly limits in biomedical applications (Mafuné et al. 2001). Biological approach provides ecofriendly non-toxic methods for nanoparticle synthesis. Varieties of organism can be used in biological approach for synthesis of nanoparticles which may include plants, fungi, yeast, actinomycetes, and bacteria (Fig. 10.3) (Konishi et al. 2007). Biological synthesis of nanoparticle as per literature is given in Table 10.1.

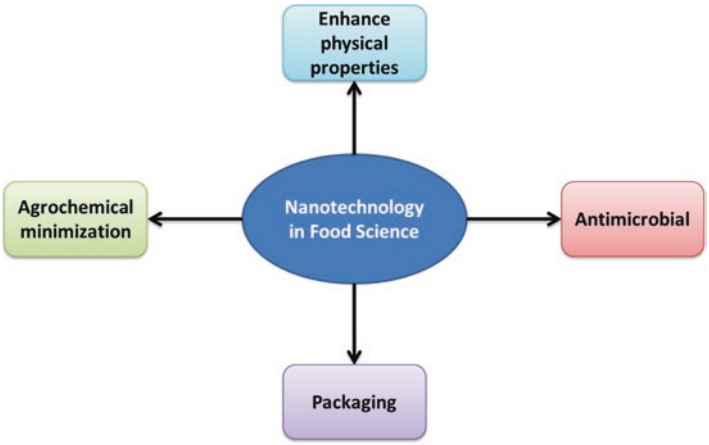

Fig. 10.2 Applications of nanotechnology in food industry. Four different aspects are being utilized in food science: agrochemical, physical properties, antimicrobial, and packaging

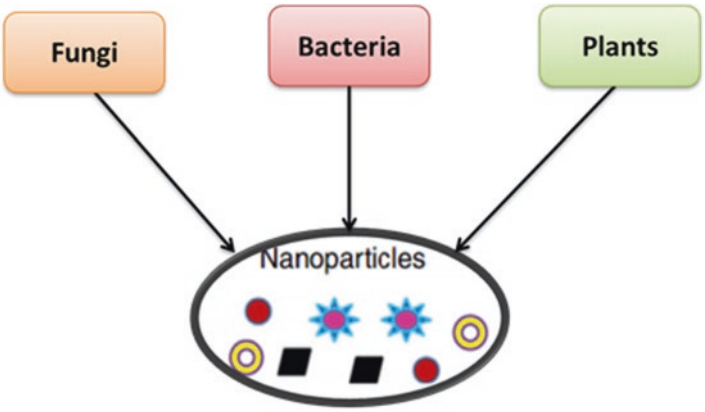

Fig. 10.3 Biosynthesis of nanoparticle. Biological synthesis of nanoparticle may be through fungi, bacteria, and pants. Each organism will produce nanoparticle of different shape and size

3 Biosynthesis of Nanoparticle Through Plants

Plant-mediated nanoparticle synthesis is quite a success and fast, cost-effective, and eco-friendly methods often used by researchers despite its pros and cons. Use of plant extracts has certain advantages like there is no problem for maintaining the culture media or preservation which is a necessary protocol in microbial culture. Plant-mediated biosynthesis of nanoparticle can be done in large scale without any contamination (Kaur et al. 2016). Various plant species have been reported to produce nanoparticle successfully (Table 10.1). Plant leaves are the site for photosynthesis, and hence they are called as food factories of plants. By taking this idea, plant leaf can

Table 10.1 Biological synthesis of nanoparticles

Sources	Name of the organisms	Localization	Types of nanoparticles produced	Size ranges (nm)	References
Plant	*Azadirachta indica* (neem)	Extracellular	Ag, Au	50–100	Shankar et al. (2004)
	Acanthella elongata	Extracellular	Au	7–20	Inbakandan et al. (2010)
	Alfalfa	–	Ti/Ni bimetallic	1–4	Schabes-Retchkiman et al. (2006)
	Simarouba glauca leaf	Extracellular	Au	10	Thangamani and Bhuvaneshwari (2019)
	Xanthium strumarium leaf	Extracellular	Pt	22	Kumar et al. (2019)
	Mirabilis jalapa leaf	–	ZnO, Ag	19.3–67.4	Nadeem et al. (2019)
	Avena sativa (oat)	Extracellular	Au	5–85	Armendariz et al. (2004)
	Aloe vera	Extracellular	Au	50–350	Chandran et al. (2006)
	Black tea leaf	–	Ag/Au	–	Begum et al. (2009)
	Capsicum annnum	Extracellular	Ag	10–40	Li et al. (2012)
	Carica papaya	Extracellular	Ag	60–80	Mude et al. (2009)
	Geranium leaves plant extract	No	Ag	16–40	(Shankar et al. 2004)
	Ethanolic extract of marigold flower	Extracellular	Ag	5	Kaur et al. (2011)
Fungi sources	*Aspergillus clavatus*	Extracellular	Ag	10–25	Verma et al. (2010)
	Aspergillus flavus	Intracellular	Ag	8.92 1.62	Vigneshwaran et al. (2007)
	Cladosporium cladosporioides	Extracellular	Ag	10–100	Balaji et al. (2009)
	Fusarium oxysporum	Intracellular	Au	20–40	Ahmad et al. (2003)
	Verticillium sp.	Intracellular	Ag	25–12	Mukherjee et al. (2001)
	Aspergillus fumigatus	Intracellular	Ag	5–25	Bhainsa and D'souza (2006)
	Schizosaccharomyces pombe	Intracellular	CdS	200	Dameron et al., (1989)
	Fusarium oxysporum and *Verticillium* sp.	Intracellular	Magnetite	20–50	Bharde et al. (2006)
	Penicillium brevicompactum	Intracellular	Ag	23–105	Shaligram et al. (2009)
	Trametes trogii	–	Ag	–	Kobashigawa et al. (2018)

(continued)

Table 10.1 (continued)

Sources	Name of the organisms	Localization	Types of nanoparticles produced	Size ranges (nm)	References
Yeast	Yeast strain MKY3	Extracellular	Ag	2–5	Kowshik et al. (2002)
	Candida glabrata	Intracellular	CdS	200	Dameron et al. (1989)
	Schizosaccharomyces pombe	Intracellular	CdS	200	Dameron et al. (1989)
Bacteria	*Pseudomonas stutzeri*	Intracellular	Ag	200	Klaus et al. (1999)
	Lactobacillus strains	Intracellular	Ag, Au	No	Nair and Pradeep (2002)
	Escherichia coli	Intracellular	CdS	2–5	Sweeney et al. (2004)
	Klebsiella pneumoniae	Extracellular	Au	5–32	Klaus et al. (1999)
	Bacillus cereus	Intracellular	Ag	4–5	Babu and Gunasekaran (2009)
	Bacillus licheniformis	Extracellular	Ag	50	Kalishwaralal et al. (2010)
	Brevibacterium casei	Intracellular	Au, Ag	10–50	Kalishwaralal et al. (2010)

Extracellular and intracellular biological synthesis of gold, silver, and other nanoparticles from different sources such as plants, fungi, bacteria, yeast, etc. is presented

be utilized to synthesize silver nanoparticles. Many reports are available for synthesis of nanoparticle by using plant leaf extract (Sripriya et al. 2019). In addition, other parts of plant like flower have been utilized to biosynthesize the silver nanoparticle.

4 Biosynthesis of Nanoparticle Through Bacteria

Bacteria can be exploited to synthesize nanoparticle. They possess certain characters like fast growth and rapid reproduction and hence are frequently used in genetic manipulations. The production of nanoparticle through bacteria may be of two different approaches: intracellular or extracellular. Most of the prokaryotic bacteria (*Escherichia coli*, *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, *Plectonema boryanum*, *Salmonella typhus*, *Staphylococcus currens*, *Vibrio cholerae*) could be utilized to produce metal nanoparticles. An overall list of bacteria and their type of nanoparticle synthesized is given in Table 10.1. Gold nanoparticles are being also synthesized by bacteria even though the exact pathway of gold nanoparticle synthesis is not known. Some researcher, however, had reported involvement of electron shuttle and enzymatic metal reduction process in gold nanoparticle synthesis (Mukherjee et al. 2002; He et al. 2011), while in bio-reduction process by the bacteria, NADH and NADH-dependent enzymes play a major role in gold nanoparticle synthesis.

5 Biosynthesis of Nanoparticle Through Yeast

Silver nanoparticles can be produced by the yeast during the log phase growth in treatment with silver solution (Kowshik et al. 2002). Yeast utilized different metabolites such as terpenoid, phenols, amines, amides, proteins, alkaloids, or other reduction substances for production of nanoparticles (Kouzegaran and Farhadi 2017). Use of yeast as biosynthesis of nanoparticle is usually taken up due to their rapid growth and simple culture techniques, and it can be used in mass production of nanoparticles. Most importantly, the production of nanoparticle by the yeast can be explained by the presence of membrane-bound structure (cytosolic) oxidoreductase and quinones. pH is one of the criteria which directly affects the activity of enzyme oxidoreductase. Increased pH of the internal environment of yeast could activate the reductase enzymes, thereby reducing the metal ions to form the nanoparticles (Salunke et al. 2015).

Likewise biosynthesis of nanoparticles can be through various other living organisms such as fungi, algae, actinomycetes, and virus.

6 Status on Food Nanotechnology

Through intervention of nanotechnology, the extensive food additives, i.e., synthetic amorphous silica (SAS), is used in powdered food products (E551), as a clarifying agent for drinks, free-flow and anti-caking agent to enhance food stability during processing and storage, improve product features or boost nutrient potency and bioavailability in foods, which is manufactured as precipitated or fumed silica and consists of aggregates of the reduced nanometre size range of main particles. The silica nanoparticles (Dekkers et al. 2013) are use as anti-caking agents, such as calcium silicate, sodium aluminosilicate, dicalcium phosphate, sodium ferrocyanide, and microcrystalline cellulose, along with SAS.

Another important food additive is titanium dioxide (E171) used as pigment to improve white colour such as dairy and candy products (Weir et al. 2012). TiO_2 is also used in a variety of non-white ingredients, including dried vegetables, nuts, seeds, soups, and mustard, as well as beer and wine, as a food additive and flavour enhancer. A study reveals that dietary intake of titanium dioxide is significantly increased, and as a result, 56% of titanium oxide was found in common food products in nanosize range (Weir et al. 2012). Nevertheless titanium dioxide nanoparticles can be used as protective agent against harmful microorganism like foodborne pathogens, and the nanoparticles combine with other compounds or elements such as nickel oxide and cobalt. As per EFSA 2016 report, the titanium oxide (E171) is recommended as one of the safety food and feed additives (EFSA 2016).

Iron oxide nanoparticles are also used as efficient food additive agents as source of iron. The iron particle is reduced in such a way that the particle size will be less and simultaneously increases the specific surface area, solubility of the particles as

well as the bioavailability of the poorly acid-soluble iron compounds. Moreover iron oxide nanoparticles may also be useful for strengthening certain foods with iron (Hilty et al. 2011).

7 Status on Agriculture Nanotechnology

Nanotechnological intervention in agriculture in the form of nanopesticides, or nano-agrochemicals, is applied to significantly improve the efficacy over the standard formulations during the agricultural practices (Gogos et al. 2012; Joseph and Morrison 2006; Kah 2015; Kah et al. 2013; Sarkar et al. 2015). This formulation can be achieved by adding the reduced nanosize ingredients along with other nano-range compounds mixing or incorporating them into solid-liquid or polymer nanocapsules (Frederiksen et al. 2003). A chitosan-based nanoencapsulates, polysaccharide-derived chitin were some of the recent advancement of nanoparticle use in agrochemicals (Kashyap et al. 2015).

A study reveals that high load ability of the nanocapsules along with the gradual release of the fungicides like carbendazim and tebuconazole could reduced (negative) impact on crop development (Campos et al. 2015). As per another study carried out by Liu et al. (2006), controlled release of pesticide validamycin and herbicide 2,4-dichlorophenoxyacetate, from porous hollow silica, has been assessed (Liu et al. 2006).

Nano-emulsions based on surfactants were assessed as an efficient delivery system for the beta-cypermethrin pesticide (Wang et al. 2007). An efficient economic cost-effective approach to control fruit pests is formulated which includes reduced doses without loss of efficacy, nano-gels containing pheromone methyleugenol (Bhagat et al. 2013). Another group of researchers studied about the applicability of the natural occurring nanosize ashes and inorganic metal nanoparticles for insecticidal, antimicrobial, or antifungal characteristics (Sonkar et al. 2012; Stadler et al. 2010; Yildiz and Pala 2012).

Nanoparticles covered with polyethylene glycol and loaded with essential oils of garlic were researched to manage pests of the stored product (Yang et al. 2009). The potential application nanotechnology can boost the crop production significantly as well as food quality by protecting crops and food products from pests (Kole et al. 2013; Mondal et al. 2011; Wang et al. 2012). Nanotechnological intervention can possibly decrease the amount of fertilizer used along with root nutrient uptake and water transportation (Pandey et al. 2010, Martínez-Fernández et al. 2016).

8 Toxicological Fundamentals and Risk Assessment

Nanotechnological application studies on the toxicity of the nanomaterials and its effect on biological systems have most crucial thing to research. As the nanomaterials are linked with structural arrangements, the nanomaterial toxicity is difficult to

predict. Indeed, although in bulk some materials are non-toxic, they might be in the nanoscale ("Nat Nano" 2011).

Due to the more complexity of the biological systems, nowadays the toxicity of the nanomaterials is usually considered to be declined (Franci et al. 2015; Krishnaraj et al. 2016; Kwak and An 2016; Martinez-Gutierrez et al. 2010; Panáček et al. 2009; Rai et al. 2009; Seitz et al. 2015). Therefore, the more complicated the cell/organism is, the less susceptible it is considered to the poisonous impacts of AgNPs. There is no such distinction, however, in some research (Greulich et al. 2012; Matveeva et al. 2006; You et al. 2011). However, comparative analysis of the toxic effects of a single silver nanoparticle on different complexity levels of biological systems is scarce.

9 Future Prospects and Conclusion

In order to promote agriculture and food industry, there has been significant increase in the use of nanoparticle against the conventional chemical products. Nanoparticles for delivery of particular drugs in plant pathogen therapy will be at its helm in near future, and the unique property of possessing such as site specific, targeted delivery, and longer retention time are of great interest for future scope. Nanoparticle treatment against the fungus of plants is showing promising and emerging area. It is further necessary to investigate on the phytotoxic action of nanoparticles and their exact metabolic pathway where different metabolites may be taking role as synergistic effect inculcates with environmental factors. Molecular-level studies of every sphere are highly commendable, and future research should try to solve the problems of comprehensive interaction between the plants and other abiotic factors by taking nano-biotechnology and genetic manipulations (Matsunaga et al. 2007).

Biosynthesized nanoparticle is one of the best options to replace the agrochemical products such as chemical fertilizers, pesticides, and other toxic chemical substances. This biological nanoparticle could be effectively utilized against the plant pathogen to protect various crop diseases. Environmental threat and global warming due to the output from agriculture sector may be minimized in far extent by exploiting biosynthesis of nanoparticles. Thus it can be said that nanoparticle in agriculture sector will help the farmers to boost their income more effectively and food safety and food security will have positive impact. Gold, silver, and other metallic nanoparticle is the replacement for the current agrochemical products. Finally, use of nano-biotechnology in agriculture and food industry is the need of hours, and it deserves all our attention to boost the farmer's income for sustainable development.

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Chapter 11
Nanoparticles in Biomedical Applications

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Contents

1 Introduction

A nanoparticle (NP) is a dynamic space for research and has accomplished a decent position in technology developments due to its exceptional characteristics (physico-chemical). Properties like electrical conductivity, melting point, thermal conductivity, light absorption, scattering, wettability and catalytic activity are resulting in improved enactment over their bulk structures. NPs are in diameter below 100 nm (Laurent et al. 2010) and are mostly divided into different assemblies on their basis of their biochemical properties, size and morphology. Few important classes of NPs are carbon-, metal-, ceramic-, semiconductor- and polymer- and lipid-based NPs as

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shown in Fig. 11.1, on the basis of their biochemical and physical characteristics. Carbon-based NPs are carbon nanotubes (CNTs) and fullerenes, in which fullerenes contain nanomaterials which are designed as a hollow cage in globular shape like allotropic type of carbon. Fullerenes have offered remarkable commercial significance because of their great strength, versatility and structure. Their electron affinity and electrical conductivity also play major role in commercial applications (Astefanei et al. 2015). Fullerenes hold carbon units that have penta- and hexagonal arrangements, and each carbon in unit has sp^2 hybridization. CNTs are the carbon-based NPs that have tubular and elongated structure in the diameter range of 1–2 nm (Ibrahim 2013). CNTs are basically resembled to rolling sheet of graphite. These great rolling sheets are called due to their number of partitions (walls) present in NPs, for example, single-walled contains single, double-walled contains double and multi-walled nanotube contains many walls (Fig. 11.2). CNTs are broadly synthesized by carbon as precursors, deposition and vaporization by laser via graphite or by electric arc on metals. Recently, CNTs are manufactured by chemical vapour deposition method (Elliott et al. 2013). Metal-based NPs are virginally prepared by the metal precursors. The NPs of copper, silver and gold come in a wide absorption spectrum which lies in the electromagnetic range in the UV- visible band.

Metal NPs catch applications in numerous research capacities due to their cutting-edge optical properties. The covering of gold NPs is extensively castoff in the specimen for SEM, for the improvement in the electronic current that aids to gain a great value for SEM pictures. Ceramic-based NPs are nonmetallic inorganic

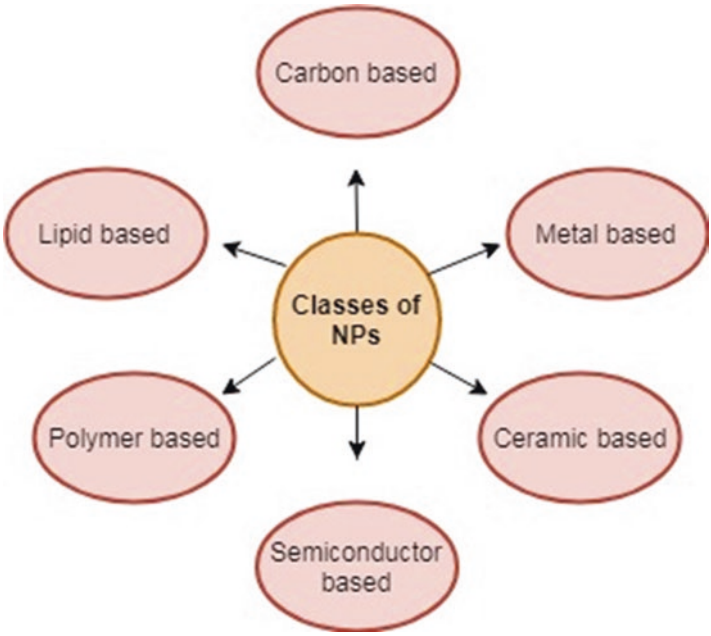

Fig. 11.1 Classification of NPs on the basis of their chemical and physical characteristics

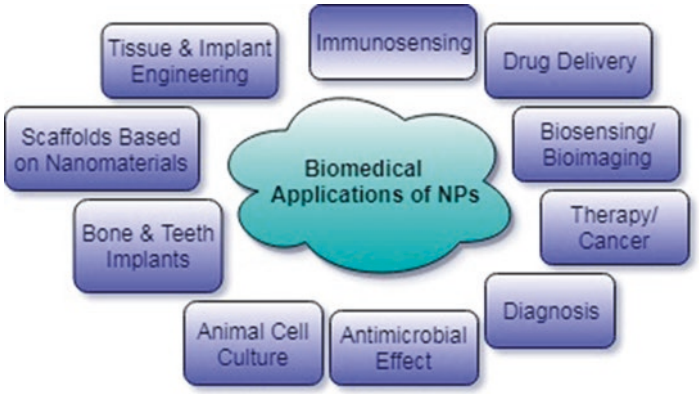

Fig. 11.2 Various biomedical applications of NPs

entities, manufactured via heating and cooling successively. Ceramic NPs are being used in thick, amorphous, hollow or porous, crystalline structures (Sigmund et al. 2006). Semiconductor materials have properties in between nonmetals and metals. Semiconductor NPs have broad bandgaps and consequently displayed significant alterated properties in tuning of bandgaps. Thus, NP is a much significant material that is being used in electronic devices, photo-optic including photocatalysis (Sun 2000). Polymer-based NPs are usually based on organic NPs as generally they are nanocapsular or nanosphere designed (Mansha et al. 2017). Polymeric NPs can be functionalized readily and, therefore, have numerous applications (Abouelmagd et al. 2016). The lipid-based NPs comprise of lipid's structure and are efficiently being used in several applications. These NPs are globular figure having a thickness range in between 10 and 10^3 nm. The lipid NPs have a dense core consists of matrix having molecules of lipophilic nature in soluble form. Surfactants are the stabilizers of the outer part of these NPs (Rawat et al. 2011).

2 Applications of Nanoparticles

NPs have exclusive properties that are being used in biomedical applications. One of the important biomedical applications is targeted drug delivery which targets anticancer drug delivery to the site-specific tumour and escapes damage to the healthy surrounding cells. Recently, the main source of materials that is magnetic is iron oxide NPs, which are being in use for anticancer drug delivery at specific and targeted areas (Berry and Curtis 2003). ZnONPs and AuNPs are also used in directed drug transport. Another main NP biomedical application is treatment of magnetic hyperthermia. The treatment of magnetic hyperthermia tumors by giving temperature above 42 ºC. As this method precisely targets the cancer cells and safeguards the surroundings tissues, it is far better than chemotherapy. Presently, the iron oxide NPs (Fe_3O_4 NPs) are

the leading NPs in the usage of cancer treatment and drug delivery (Pankhurst et al. 2003; Laurent et al. 2011), though other nanosystems like bimetallic NPs Cu–Ni and Fe–Co and magnetic NPs, e.g. Ni–Co_2O_4, Mn–Fe_2O_4 and Co–Fe_2O_4, have likewise been examined. The practice of magnetic NPs for bioimaging methods like MRI and computational tomography is alternative significant biomedical application, as contrast agents (Berry and Curtis 2003; Pankhurst et al. 2003; Tartaj et al. 2003).

In MRI, Fe_3O_4 NPs are the key substitute being examined to substitute gadolinium chelates that are now in usage. Additional magnetic NPs which are being examined as potential contrast agents comprise Fe–Pt, Co–Fe_2O_4, Fe–Co, Mn–Fe_2O_4 and Fe–Ni. Photoablation remedy practices materials that are light sensitive to abolish diseased area like cancerous tumours. Many NPs such as Fe–Pt, Au, TiO_2, ZnO and Ag have been examined to likely use in therapy (Linsebigler et al. 1995; Dougherty et al. 1998; Allison et al. 2006; Kim et al. 2013a, b; Yin et al. 2013; Muddineti et al. 2015). Biosensors have appeared as a significant biomedical application in sensing and detecting a range of biomolecules. Many NPs like CeO_2, TiO_2, Au, and Fe–Pt have been examined for potential usage in biosensors. This chapter explains important NP applications in biomedical particularly immunosensor, drug delivery system, biosensing and bioimaging, cancer therapy, antimicrobial activity, animal cell culturing, bone and teeth implanting, nanomaterial-based scaffolds and tissue and implant engineering.

3 Role of Nanotechnology in Biomedical Applications

3.1 In Drug Delivery

Nanoparticles confirm the strength and bioavailability of a drug, thus leading to develop nano-medical approaches for delivering drugs. Various factors, such as drug leakage into blood vessels, affect the strength and bioavailability of drug. Nanotechnology supported approach in delivering drug aids in improving permeation and directing of drug along with its controlled release (Ringe et al. 2004). Gene therapy is an advance approach (Langer 2001) which resulted in efective treatment; also it decreases the harmful outcomes and drug amount with better strength of drug. Especially it is very much applicable in chemotherapy against cancer, which adjoining healthy cells are more important than to selectively transport the drug to neoplastic cells.

Nanomaterials were used in targeting approaches which leads to higher intracellular drug concentration in tumour cells, thus preventing the healthy cells from toxicity (Allen 2002). Non-modified nanoparticles are employed in passive targeting. The disadvantage of passive way is getting trapped of nanoparticles in the reticuloendothelial circulating system. Targeting passively uses the different path for tumour vasculature, retention effect (EPR) and permeability enhancement explicitly due to which penetration of nanoparticles occurs via minor capillaries which permits accumulation of drug at specific places. Design's flexibility and big

surface space help in an organized drug release (Liu and Webster 2007). The key parameters of releasing drug are size and dispersion of nanoparticle (Gorner et al. 1999). In releasing of drug and delivery of gene, many nanoparticulate methods such as PEG; silica; biodegradable chitosan; gelatin, dextran, etc. (Saji et al. 2010); and polymers that are non-biodegradable like polyacrylamide, polyphosphazene derivatives, PMMA, etc. were used (Ravi kumar et al. 2004).

By the process of diffusion and endocytosis, nanoparticles can move via cell membrane's lipid bilayer. Additionally, they can move into the cells by connecting via immunoglobins like antibodies. Nanoparticles when connected to antibodies/surface receptors of cancer cells, the nanoparticles which are functionalized should also been castoff for directed access into cancer cells (Doubrovsky et al. 2010). The 20 nm gold nanoparticles are conjugated with many peptides which are targeted to deliver functionalized nanoparticles, which then targets the nucleus and also penetrates the cell membrane. For the medical analysis and cure of cancer, the various functionalized nanoparticles are used for delivering drug and directed biomarkers (Daraee et al. 2014). Many cytoplasmic enzymes especially which are proteolytic regularly hinder the drug delivery system. Drug and gene delivery system often use graphene oxide which acts as a transporter.

Various functional groups of graphene oxide present such as OH and COOH permit the conjugation with biomolecules and polymers. The functionalization of graphene oxide with polymer (which is cationic in nature) such as polyethylenimine is a good approach. Gene delivery system uses grapheme oxide because it efficiently interacts with DNA and RNA's negative charge carried by phosphate ions. So graphene oxide aids in transfection, reduction in cellular toxicity and improvement in selectivity of cell. Delivering of protein (anti-apoptotic) Bcl-2-targeted doxorubicin (anticancer drug) and siRNA in conjugation with graphene oxide functionalized with PEI gives an elevated efficiency in transfection and less PEI cellular toxicity with increased efficiency in anticancer activity, displaying a synergistic effect (Priyadarsini et al. 2018). In debt of unique properties like chemical, electrochemical, optical, electrical and electronic properties of graphene and its derivatives (Banerjee 2018) and the bio-functionalization of graphene-based nanomaterials with various biomolecules used in different biomedical applications. Apart from graphene oxide containing functional groups and furthermore having large surface area with basic sp^2 planar structure, it also gives good capacity of loading and biocompatibility along with great solubility. Multimodal graphene oxide can be formed by using modest chemical conjugation or physico-absorption of proteins and biomolecules along with polymers onto graphene oxide. Graphene oxide is a proficient nano-carrier in anticancer drug (water-insoluble) delivery system.

Graphene oxide with polyethylene glycol-grafted (N-terminal) conjugated with an anticancer drug SN38 which is water insoluble via adsorption non-covalently was selectively directed against tumour. The occurrence of π-electrons in delocalized form on the exterior of graphene helps in conjugation of anticancer medicines (aromatic) via π–π stacking. For careful assassination of tumour, nano-graphene oxide (NGO) PEGylated sheets along with doxorubicin in conjugation with antibody have been used. The doxorubicin's quinone structure attaches to graphene

oxide through π–π relations, while H-bonding is seen among the NH_2 and OH groups of doxorubicin and also between the OH and COOH groups in graphene oxide. There is a pH impact on loading the drugs and their kinetic release. Neutral pH was best for full loading of drugs; however at pH 2, around 70% of drug was released which is acidic in nature. In this acidic condition, the NH_2 group of doxorubicin becomes protonated which leads to the dissociation of the H-bonding; henceforth releasing of drugs occurs. For the improvement of cellular acceptance of doxorubicin efficacy, gold nano-clusters should be used with reduction of graphene oxide, resulting in suppressing of tumour's progression.

3.2 Biosensing/Immunosensing

Research and development in different frontiers of nanobiotechnology aims to develop highly functional biosensors and nanosized microchips. Graphene has been employed for advancement of various electrochemical biosensors by virtue of its large surface area, high electron transfer potential and hence excellent electrical conductivity. Detection of various biomolecules like proteins, growth factors and nucleic acids by monitoring changes in an electrical signal by different field-effect transistor biosensors has become possible by utilization of graphene. For in situ sensing of biomolecules, graphene-field-effect-transistor based biosensors have been massively explored for the detection of nucleic acids, proteins, etc., and the growth factors have been effectively confirmed by using appropriately functionalized graphene derivatives with nucleic acids, aptamers and carbohydrates for monitoring target-specific changes of electrical signal with high signal-to-noise ratio (Park et al. 2009). Due to graphene's fluorescent quenching property, GO-labelled fluorescent-marked ssDNA have been utilized for manufacturing of DNA/nucleic acid biosensors. ssDNA florescence is quenched by GO. The formation of double helix from this ssDNA upon coming in contact with target complementary DNA sequence displaces GO from this ssDNA strand, resulting in fluorescence recovery. Various biosensors for detection of DNA have been developed utilizing graphene (Shen et al. 2012). For instance, nitrogen-doped graphene FET biosensors have been developed for detection of VEGFs and catecholamines like dopamine, epinephrine and norepinephrine (Kwon et al. 2012). For simultaneous detection of various metabolites of human digestion, for example, uric acid and ascorbic acid, a few graphene-based sensors have been created (Suvarnaphaet and Pechprasarn 2017). Electrical signal from electrogenic cells like cardiomyocytes has been detected by using FET biosensors based on CVD graphene. Biosensors based on graphene have also been constructed for detection of hormones, proteins, adenosine triphosphate (ATP), fungal toxins and toxic metal ions. GO biosensors were also created for estimating the activity of many enzymes like trypsin, thrombin, caspase-3, metalloproteinase and DNA helicase (Chung et al. 2013). Graphene-based biosensors have also been developed for detection of pathogens like Huang et al. (2011) who developed a highly selective nanoelectronic biosensing device for the

recognition of *Escherichia coli* (*E. coli*). In addition, for cancer diagnosis, also graphene has been utilized as immobilization support for primary antibodies against prostate-specific antigen (Qu et al. 2011). Further, graphene has also been utilized for the advancement of biosensor in detecting lipopolysaccharides (LPSs). Limulus amebocyte lysate (LAL) assay is the most frequently used enzymatic assay for LPS detection. Graphene-based biosensors resulted in sensitive, selective and rapid methods to sense various analytes.

3.3 Bioimaging

GO having diverse functional groups can conjugate with fluorescent dyes, so it has been utilized for bioimaging. Next to this, GO displays high absorbance and fluorescence characteristic in the NIR spectrum, and its properties can be controlled by changing rate of reduction, size, pH and time of its development. Cheng et al. (2012) demonstrated simultaneous imaging and drug delivery using GO under mild thermal annealing which showed blue fluorescence due to the development of sp2 and oxidized domain. Annealing induces phase transformation in GO which enhance oxygen diffusion, resulting in the formation of nanosized (1–1.5 nm) graphitic domains responsible for the blue photoluminescence.

Additionally, nanomaterials have been used for optical and MRI as contrast agents. Utilization of nanomaterial based agents for optical imaging has improved stability in both in vitro and in vivo systems, protection from photobleaching, large quantum yield, strong absorbance, non-toxic, and near infrared (NIR). Nanoparticles with NIR excitation and emission find excellent use in imaging of deep cancerous tissue. For example, liposomes including silica labelled with dye, quantum dots and gold nanoparticles (AuNPs) have been exploited as optical contrast agents. For silica/polymer labelled with dye, the source of fluorescence is dye itself encapsulated within matrix of nanoparticles. Though gold and silver are non-fluorescent, they exhibit surface plasmon resonance. Optical contrast agents having high sensitivity, stability and clinical safety, are in great demand.

Recently, Qdots have attracted great attention. Integration of several imaging techniques like fluorescence, X-ray, CT and MRI into a single nanoparticle probe makes for better cancer imaging. Due to low reflexivity values and potential toxicity, they have been frequently used as MRI contrast agents. Water-soluble endohedral metallofullerenes have also been used as MRI contrast agents. Further, superparamagnetic iron oxide nanoparticles have been proposed a good choice as MRI contrast agent due to their biocompatibility and biodegradability. Moreover, research is going on for use of CNTs, ultra-superparamagnetic iron oxide nanoparticles (USPION) and paramagnetic liposomes as new contrast agents for MRI. As gold nanoparticles (AuNPs) possess interesting size-dependent chemical, electronic and optical properties, hence, they are used for nanomedicine purpose. In addition to it, for different biomedical purposes including cancer imaging and therapy, AuNPs permit numerous surface modifications which results in its reaction

with lots of chemical and biochemical vectors. For imaging contrast enhancement, surface plasmon resonance (SPR) properties of AuNPs can be tuned to make them best agent. Various Au nanoforms like nanorods, gold colloids and composite or hollow nanoforms have been emphasized for biomedical applications (Ghosh et al. 2008). A very simple method, galvanic replacement reaction between silver nanocubes and gold chloride can form novel nanoforms, Au nanocages. Enhanced spectroscopic images have been reported in tissues having nanocages of gold (Skrabalak et al. 2007). Prediction and optimization of image contrast properties is a prerequisite for efficient exploitation of nanostructures for imaging. Besides this, for photothermal and radiotherapy, AuNPs have been intensively used (Huang et al. 2007). Tong et al. demonstrated a firm basis to develop a targeted photothermolysis for cancer therapy using gold nanorods whose effectivity got enhanced under fs pulsed excitation leading to high absorption in NIR spectrum and transformation of photothermal energy.

The wavelength of plasmon absorption gets shifted due to use of surface-modified gold nanoparticles for biosensing purposes. Use of nanomaterials for development of biosensors resulted in their improved sensitivity, specificity and reliability. Pandey et al. (2008) described that the area of biosensors based on nanomaterials (e.g. nanowires, nanotubes, nanoshells, etc.) is very broad which involves electrochemical, electrical and optical biosensors along with field-effect transistors. Surface-enhanced Raman scattering (SERS) assisted with gold nanoparticles led to an effective determination tool for specific molecules. By conjugating a label molecule on their surface, a SERS nanotag has been created for detection. Moreover, they have been exploited for other biomedical purposes, e.g. as an effective means to test pregnancy based on micro-albumin (Viroj et al. 2007).

As Qdots possess physical dimensions smaller than Bohr exciton radius, they have been employed for imaging cancer cells and tissues. Due to their large Stoke's shift value, they prove advantageous to minimize the background signal, thus resulting in sensitive detection. Moreover, their potential permits the simultaneous sensitive determination of cancer-specific surface receptors by a single excitation. Further, it diagnoses the early stages of cancer (Yezhelyev et al. 2007). They have also been applied to label and track the cell and pathogen and develop FRET-based sensors. Besides that, they have played crucial part to diagnose biomolecules in real time even at the single molecule level. Exchange of ligand and polymer coating has been used to modify their surface.

Wang et al. (2011) described the development of various graphene based optical/electrochemical biosensors, electronic devices, mass spectroscopy and bioimaging due to its and its derivatives unique characteristics, e.g. electronic, optical, chemical, electrochemical and electrical properties. Further, for in situ sensing of biomolecules, DNA, a nanoconjugate of graphene derivative with oligonucleotide has been employed. Moreover, using graphene, its derivatives tagged with nucleic acids, aptamers and carbohydrates have been employed to determine nucleic acids and proteins with high signal-to-noise ratio (Park et al. 2012; Ohno et al. 2010; Kwon et al. 2012;, Mao et al. 2010; Tang et al. 2010). Kwon et al. (2012) described a nitrogen-doped graphene FET biosensor to sense vascular endothelial growth factor (VEGF). Similarly, Mao et al. (2010) immobilized anti-IgG onto the surface of ther-

mally reduced graphene oxide (TRGO) through gold nanoparticles and sensed the precise recognition site for the binding of immunoglobulin G. Consequently, for biomaterials like hormonal catecholamines and heavy metals, different processes like DNA hybridization, protein binding events and field-effect transistors (FETs) based on GO have been proved as an effective tool. Further, for imaging of cells in vivo, MRI signals have been amplified using magnetic NPs in association with GO. Chen et al. (2011) described the conjugation of amino dextran-coated Fe_3O_4 NPs with GO for MRI. Hong et al. (2012) reported the use of radio-labelled GO for imaging of cancer cells using positron emission tomography (PET). Besides this, for innovative biosensing techniques, graphane, a fully hydrogenated form of graphene, has been discovered as a novel nanomaterial (Tan et al. 2013). In comparison to graphene, graphane has emerged as better option due to its electrochemical behaviour towards oxidation/reduction of different biomolecules, e.g. ascorbic acid, dopamine and uric acid.

3.4 In Therapeutics

Cancer medical care employs a borderline invasive methodology in the form of photothermal (PTT) medical care which exploits physiological effects produced by photothermal agents to destroy cancerous tissue. PTT exploits NIR due to its characteristic to penetrate in tissues in combination with photothermal agents, which transform NIR radiation into heat. Using heating effect, cancerous tissue is destroyed as the temperature reaches to 42–43 °C, while healthy tissue does not get affected. Niidome et al. (2016) reported the use of different nanoforms of gold, i.e. nanoshells, nanorods, nanocages and nanourchins with NIR, for efficient photothermal medical care. The plasmonic absorption is often adjusted by optimization of surface properties, form and size of nanoparticles (Singh et al. 2018).

LAT-1 ligands like L- and D-dopa were used as reducing and capping agents in the preparation of AuNPs for efficient targeting of the massive neutral aminoalkanoic acid transporter LAT-1 (Ong et al. 2017). A great attention has been caught up by LAT-1 transporter due to its additional utility as a biological marker for imaging and therapeutics of human malignancies. Gold nanoparticles functionalized with L-dopa or D-dopa gave promising leads to cell lines like MDA-MB-231, MDA-MB-468, MCF-7 and MDA-MB-453 in comparison to non-targeted management.

Xue et al. (2017) reported formation of golg nanosheets (AuNShs) and gold nanocages (AuNPs) utilizing a biotemplate named lanreoite acetate (Lan). The temperature raised to 54.3 °C and 46.8 °C for AuNCs or AuNShs, respectively, upon NIR irradiation of 808 nm at 0.8 Wcm^{-2} for every 30 s in in vitro system. They inhibited the growth of HeLa cells upon irradiation by 86.26% for AuNCs and 75.56% for AuNShs, hence proving them as an efficient photothermal tool.

AuNCs and AuNShs along with NIR irradiation led to strong inhibition of tumour growth in murine model. Gold nanocages and nanoshells were injected and irradiated in mice. Temperature of tumour surface raised to 48.3 °C for AuNShs within 5 min and more than 50 °C for AuNCs within 3 min upon injection of AuNCs

or AuNShs and irradiation in mice. Consequently, tumour weight decreased considerably in these groups after 12 days, while control groups revealed no noteworthy modifications. Injection of AuNCs and AuNShs resulted in tumour inhibition by 86.65% and 72.39%, respectively, thus establishing the effectiveness in PTT. Manivasagan et al. (2018) studied the effect of gold nanorods tagged with chitosan oligosaccharide (COS) and lipoic acid (LA) to diminish the cytotoxicity caused by CTAB, which resulted in outstanding PTT. Moreover, gold nanomaterials (25 g/ml) injected and irradiated with NIR in tumorous mice culminated into complete tumour disapperance and normal tissue reconstruction, a proof of excellent thermal response. Targeting the subcellular entity has been introduced as effective methodology to improve PTT efficacy. This protocol helps in nuclear translocation using nuclear localization sequence (NLS) and induces hyperthermia near specific organelles (Chen et al. 2018). Biocompatibility and cellular targeting have been improved by developing AuNUs coated with hyaluronic acid (HA), and nuclear translocation was enhanced with AuNUs coated with cationic NLS. Gold nanorods efficiency was found intensified under certain conditions like fs-pulsed excitation, increased efficiency of NIR absorption and photothermal energy conversion and hence provides a solid foundation for developing targeted photothermolysis for cancer therapy (Tong et al. 2007).

These AuNU@NLS@HA nanoplatforms stood firm in testing standards of high stability, good biocompatibility, precise tumour-targeting, great cellular internalization and very good photothermal activity. Moreover, experiments in in vivo and in vitro systems exhibited encouraging outcomes in suppression of primary and metastatic tumours (Chen et al. 2018). Recently molecularly imprinted polymers (MIPs) present an innovative approach in development of new PTT agents. MIPs are artificially developed antibodies with relatively enhanced specificity and chemical stability in comparison to substantial antibodies. Yin et al. (2017) synthesized AuNRs imprinted with sialic acid for specific cancer cell targeting which showed promising photothermal effect on cancerous tissue without damaging surrounding healthy tissue.

Photosensitizers have been employed in photodynamic therapy (PDT) where reactive oxygen species (ROS) are generated upon excitation by specific wavelength leading to cellular apoptosis. PDT in combination with PTT presents a promising joint therapeutic approach for treatment of cancer ailments. Combined use of nanoparticles and molecules generating ROS is a field of fascinating prospects; moreover, there is decreased therapeutic effect of drug due to too fast drug release from NPs while entering the body. Moreover, intrinsic fluorescent quenching of photosensitizers by FRET makes PDT-mediated imaging of tumour and tracing of drug even more difficult. Hence, innovative approaches for photosensitizers' loading on AuNPs are prerequisite for an efficient joint therapeutics and imaging using PDT/PTT. For example, tricarbocyanine dye, namely, indocyanine green (ICG), approved as NIR photosensitizer, unfortunately showed poor stability leading to quick clearance from blood and thereby low quantum yield. Nowadays, an anisotropic AuNP-based nanomaterial has been synthesized with encouraging results as joint PDT/PTT therapeutic agent (Liu et al. 2017). Anisotropic AuNPs were double coated with a combined layer of $CaCO_3$ and ICG, where stable $CaCO_3$ aggregates with anisotropic AuNPs prevent its fast

blood clearance, and acidic medium degradation of $CaCO_3$ layer makes ICG selective release in tumour tissue at pH 6.4 a real possibility. An effective combined anti-tumour effect has been observed in experimental setup in vivo as well as in vitro by AuNPs@$CaCO_3$/ICG upon NIR irradiation; additionally, the NP biodistribution in the tumour can also be seen using fluorescence imaging.

3.5 The Radiotherapy

Cancer treatment is done by radiation therapy which can cause harm to healthy tissues/organs by diffusion and have less specificity towards cancerous cells (Cheng et al. 2018). Earlier, the effect of radiation has been increased by use of nanoparticle-based radiosensitizers as they have the ability to generate electrons which can specifically target on cancerous/tumour cells without damaging nearby healthy tissues. Later on there is generation of reactive oxygen species to enhance radiation-induced harm (Goel et al. 2017). It was studied that radiation sensitization increased synergistically through the contact of gold and titanium oxide within a nanostructure. Nanostructure shows more production of reactive oxygen species as compared to nanoparticles of gold and titanium dioxide alone. Radiation sensitization of nanostructure was justified by triple-negative breast cancer cells (SUM159) of mice which indicate that the tumour development was considerably decreased in mice treated with nanostructure and X-ray (Cheng et al. 2018).

Enzyme cyclooxygenase-2 (COX-2) overexpression was an indication of tumour genesis and treatment (Zhou et al. 2009; Xu et al. 2014; Karahan et al. 2018). Gene suppression has been done by small interfering RNA (siRNA), but still an interference RNA provision system is essential. Zhu et al. studied blockage of glucose transporter 1 (GLUT1) by modified 2-amino-2-deoxy-D-glucose (DG)-polyethylene-glycol (PEG) gold nanoparticles and overexpression in cancer cells. GLUT1 act as marker of tumour genesis and can transport DG into cells. Along with this, functionalization of gold nanoparticles with lysine, lipoic acid and 9-poly-D-argine (9R) was enhanced, and siRNA/9R/DG-AuNP hydrazone nanomaterials in in vitro experiments suppressed COX-2 efficiently in SGC7901 and HepG2 cells.

A method for treatment of cancer is hyperthermia in which cancer cells and healthy cells indicate symbols of apoptosis (41–47 °C) and necrosis (above 50 °C) (Milleron and Bratton 2007). Due to higher rate of metabolism, cancerous cells were more prone to heat than healthy cells (Huff et al. 2007); therefore hyperthermia was likely used to treat cancer. Hyperthermia was done by microwave, laser and radio waves, but by using magnetic nanoparticle for heating offers a non-hostile way to increase cell temperatures to a therapeutic level, diagnostic level and imagined by MRI. Initially, all magnetic nanoparticles are supplied into tumours and heated using alternating magnetic fields to attain required temperatures (Pankhurst et al. 2003). Moreover, chemotherapy or radiotherapy can also be used for the functionalization of magnetic particles. Brown relaxation and Neel relaxation techniques were used for the process of heating of magnetic nanoparticles (Cherukuri et al.

2010). This mechanism was also operative for materials which have near infrared (NIR) absorption competencies, such as gold nanoparticles (Gobin et al. 2007; Gannon et al. 2008) or carbon nanotubes (Kam et al. 2005).

Magnetic nanoparticles as hyperthermia agents were also used in vitro and in vivo as shown by many studies (Jordan et al. 1997). A glioblastoma multiforme is a type of brain cancer that was treated by aminosilane with iron oxide nanoparticles (Maier-Hauff et al. 2007). There were many MRI scans used for locating tumour. An alternating magnetic field was exposed to patients to persuade particle heating. Nanoparticles were tolerated by all patients without any difficulties. Deposition of nanoparticles was found stable for many weeks as observed by CT scans. Hyperthermic nanoparticles to cure prostate cancer were studied by related groups (Johannsen et al. 2007a, b).

3.6 In Diagnosis

Chemical and biological agents have been detected by use of gold nanoparticles. The confocal laser microscopy has gained growing attractiveness due to gold nanoparticles in medical and biological research (Wang et al. 2010). Confocal images were taken by different confocal microscopy such as fluorescence microscopy or resonance scattering or two-photon luminescence. Strong decrease in background signal was the major benefit of this technique and effects in the contrast are being improved (Daraee et al. 2014). Gold nanoparticles, carbon nanotubes, quantum dots, polymer nanocapsules, nano-HA, chitosan, dendrimers and liposomes have been broadly used for various medicinal applications. Early diagnosis and cure of disease by nanocomposites which give a promise raised area both in vitro diagnosis such as in intracellular molecular imaging, highly sensitive solution assays and molecular profiling and in vivo diagnosis such as Raman-active nanoparticles for Raman spectroscopy, magnetic nanoparticles for magnetic resonance imaging, Qdots for optical imaging, etc.

3.7 Animal Cell Culture

Due to insignificant harmfull effects on animal cells, ability of linkage, gene transfection, stem cell differentiation, neural differentiation, graphene based nanomaterials used as scaffolds for culturing cells and engineering of tissues (Park et al. 2011; Wang et al. 2012; Chen et al. 2012a, b; Ryu and Kim 2013; Kim et al. 2015; Lee et al. 2015; Garcia-Alegria et al. 2016; Kumar and Chatterjee 2016; Bouzid et al. 2016; Shin et al. 2016; Akhavan 2016; Shadjou and Hasanzadeh 2016). Stem cells of mouse embryo differentiate into haematopoietic cells and significantly increased by using GO-coated substrates. Haemogenic endothelial cells differentiated from

haemangioblasts during haematopoietic arrangement were measured as a crucial stage. Furthermore, graphene oxide also increases separation of embryonic stem cells of humans to blood cells. Likewise, it was informed that the length and number of neurite both improved in neuroblastoma cells of humans when scattered on graphene, compared with control glass substrate (Bouzid et al. 2016; Lee et al. 2015). Recently, three-dimensional printable graphene was a biocompatible elastomer reported for growth of mesenchymal stem cells and differentiation of neuronal and glial genes (in vitro) and has encouraging stability at least 30 days (in vivo) (Jakus et al. 2015). Graphene- and graphene-based nanocomposite, e.g. graphene oxide, reduces graphene oxide nanosheets that showed valuable inhibition of *E. coli* bacteria growth due to synergistic effect on their surface during insignificant cytotoxicity (Li et al. 2014a, b). For instance, a few studies have shown that the GO have more antibacterial growth as compared to reduced graphene oxide as graphene oxide provides surface for attachment and growth of cells as defined by the incentive of bacterial increase (Hegab et al. 2016). Information showed that methodically washed and greatly purified GO showed least antibacterial properties against both Gram bacteria whether positive or negative. Deoxyribonucleic acid, proteins, membranes, etc. are different components of cells which initiate a sequence of interactions (nanomaterial–bacterial). These interactions depend on shape, size, hydrophobicity, roughness, functionality, dispersibility, concentration, purity and colloidal energies of the graphene-based nanomaterials.

3.8 Tissue and Implant Engineering

In this field, research and development enhance the prudent tissue synthesis and osseointegration for clinical implant purposes. On the basis of hydroxyapatite (HA), bioactive surface coatings were done to decrease the implant rejection rate. An in vitro and in vivo study of nanomaterials was done in different medicinal areas (Robert et al. 2007). Presently, synthesis of tissue can be improved by nanotechnology approaches which showed alternation of biomaterial surfaces and application of nanomaterials for new implants.

3.8.1 Alteration of Biomaterial Surfaces by Nanotechnology

Nanoscale surface roughness was improved by titanium-based implants which showed that nanotopography changes cellular response via protein deposition, controlled growth of cells, better linkage and multiplication of bone cells. Binding ability of cells and proteins increased due to large surface area and surface energy (Jager et al. 2007). Modification of surface topographically and introduction of chemical molecules on a surface are the two approaches for nanoscale surface modification.

3.8.2 Alteration of Surface Topographically

Colloidal and imprint lithography allows the construction of two-dimensional and three-dimensional structures with high resolution with effective ease (Wood 2007) for biological applications as compared to traditional photolithography. In addition to lithographic techniques, there are many physical methods like nanoparticle deposition and ion beam deposition and chemical methods like anodization and acid etching discovered to create implant surfaces at nanoscale (Mendonca et al. 2008). The properties showed by nanotubular surfaces for culturing cells were better adhesion, explosion, bone matrix deposition and alkaline phosphate activity as compared to titanium surfaces (Popat et al. 2007). It was studied that bioactivity of such nanotubular surfaces increased by nanoscale hydroxyapatite (HA); HA-nanoporous titania in argon atmosphere was reported for increasing bond strength (Kar et al. 2006). Nanotubular titanium alloys were provided with low temperature in biocompatibility point of view (Saji and Choe 2009).

3.8.3 Introduction of Nanoscale Chemical Molecules on a Surface

Surface chemistry was changed by deposition of nanoparticles onto implant surfaces. Studies reported that isolated silver nanoparticles were deposited on poly(methyl methacrylate) (PMMA) bone cement and prevention of bacterial colonization by covering orthopaedic pins with Ag nanoparticles (Wagener and Biogate 2006). Copper metal showed the highest bacterial reduction rate as compared to different metal salts of silver, zinc, mercury etc. that were deposited on Ti surfaces for production of nanoscale sol–gel titania layers (Heidenau et al. 2005). Osteointegration and antibacterial effects were studied by modified titania and zirconia nanocrystal coatings (Bignozzi et al. 2008).

Three-dimensional (3D) printing in tissue and organ engineering is a novel technology used to manufacture two-dimensional graphene into a three-dimensional structure with the help of polymer, ceramics or metals to form three-dimensional columns using software related with the printer. Modern devices and sensors were improved by scientists and engineers for tissue engineering with the help of ink-based printing. The properties such as mechanically resilient, high electrical conductivity, more tensile strength, ability to resist pressure and highly bioactive which will greatly increase the graphene material adaptability for application in biomedical (Jakus et al. 2015). The properties exhibited by functional materials for 3D graphene inks are able to print rapidly and user-friendly. Necessary cell response can be attained by porosity of 3DG. Likewise, 3DG remain feasible for culturing and multiplication of human mesenchymal stem cells (MSCs). Uncontrolled abrasions decreased in organs due to insertion of graphene sheets. The properties like mechanical and thermal strength of three-dimensional nanocomposites were enhanced by integration of polyurethane/polylactic acid (Syama and Mohanan 2019). Therefore, biomaterials may be modified for application of complex tissue engineering by 3D printing to get attractive multiple functionalities besides fabricating surgery-friendly constructs (Syama and Mohanan 2019).

3.9 Antimicrobial Effects

Magnetic nanoparticles of titanium oxide, zinc oxide, magnesium oxide, chitosan, copper and silver are used as antibacterial agents for bacterial infection (Jones et al. 2008). Silver nanoparticle synthesis and antibacterial effect on GO sheets have been studied (Tian et al. 2011). GO was physicochemically characterized by using thermogravimetric analysis, X-ray diffraction, transmission electron microscopy and ultraviolet-visible and Raman spectroscopy. Antibacterial property of GO and GO–Ag against microbes was detected with the help of standard counting plate method. An antibacterial effect was shown by both GO and RGO against a variety of Gram staining bacteria (Akhavan and Ghaderi 2010). Liu et al. (2011) presented the antibacterial action of graphene by connection of bacteria to graphene sheet surface, damage of membrane that causes intracellular content leakage and oxidation of membrane components. RGO nanowalls were more toxic due to the presence of better charge transfer and sharp edges towards bacteria than GO (Guo and Mei 2014). By avoiding electron transport chain to make sure in use of adenosine triphosphate by graphene and, finally causes death of cell. The components of cell like protein, lipid and deoxyribonucleic acid are damaged due to formation of reactive oxygen species inside the cells by graphene. Formation of lipid peroxides during fatty acid oxidation breaks down cell membrane and finally results in cell death. Polymers of various graphene composites have been produced to offer antibacterial surface for various applications in biomedical field. GO with amine-containing organic compounds showed antibiofilm and antimicrobial activity against bacteria as compared to amines alone (Zarafu et al. 2018).

An antimicrobial peptide ($G(IIKK)_4I\text{-}NH_2$) was charged by GO and its constant discharge as reported by Cao et al. (2018). GO-modified surface showed three times higher bacterial growth which proposed that sufficient wettability for linkage and multiplication of bacteria were measured by GO oxygen groups. In spite of the contrary results, materials based on graphene can be used for coatings due to antimicrobial property, for wound coverings (Giulio et al. 2018), on surface of medical devices and as smart antibiotics (Karahan et al. 2018), after a systematic analysis.

3.10 Scaffolds Based on Nanomaterials

In tissue engineering analysis, scaffolds have a major contribution because they provide templates to give unique construction and growth of tissues besides structural support for particular cells. Therefore, due to sensible biocompatibility and biodegradability property of 3D porous scaffolds, its fabrication plays important role (Kim and Mooney 1998). Development of fibres ranges from few microns to micromillimetre scale by a new technique known as electrospinning under high electrical fields. Therefore these scaffolds offer more surface area and some more advantages due to topographic features of the extracellular matrix for development of latest tissues (Liang et al. 2007).

On the basis of tissue engineering in stem cell, 3D scaffolds help in regeneration and recovery of broken tissue due to their features like organic chemistry, biophysical and mechanical. These meaningful scaffolds are made due to proper management and improvement of such features. Therefore, scaffolds enhance the regenerative ability of stem cells. Vegetative cell composition and functions like self-renewal, production, and stem cell differentiation were regulated by improved biophysical and mechanical indications together with physical possessions, stiffness and structure (Chen et al. 2012a, b; Kamei et al. 2013). The various properties of stem cells were improved due to distinct structural and mechanical properties of 3D scaffolds for tissue engineering. Several important techniques like electrospinning, lithography, microfabrication and self-assembly were widely discovered to construct 3D scaffolds relevant for particular tissue uses (Kim et al. 2013a, b). Undecorated trauma and non-inheritable malformations of the bone were recovered by tissue engineering technique. Many reports showed that graphene enhances linkage, multiplication, osteoblasts and biological properties of scaffold materials.

Regeneration of the bone needs a signal for morphology, host cells for response, an appropriate carrier of this signal and a viable, well-vascularized host bed (Tiffany et al. 2012). For regeneration of tissues, features like biological compatibility, cell growth, proliferation and differentiation of scaffold material are important (Bose et al. 2012). Cell behaviour together with attachment, growth, repair, proliferation and differentiation is supported by aromatic scaffold nature of graphene and graphene oxide (Ryoo et al. 2010).

Freeze-drying technique was used for the combination of GO nanoflakes (0.5 and 1 wt. %) with gelatin–hydroxyapatite (GHA) matrix for improving mechanical strength and osteogenic differentiation in latest times (Nair et al. 2015). Osteogenic differentiation was induced by GOGHA0.5 scaffold in human mesenchymal stem cells of adipose tissue for continued culture experiments without any supplements within the medium. In medical science, biocompatible, perihable and porous graphene oxide-strengthened gelatin-HA 3D scaffold which could function as an appropriate candidate for promotion of bone regeneration. Researchers indicated that coating of GO enhanced numerous medicinal features of scleroprotein scaffold. It also increased surface structure, compressive strength and cell development (Nishida et al. 2014). For bone tissue engineering, graphene–hydroxyapatite gels are extremely strong, permeable, electrically semiconductive and biocompatible, creating them promising scaffolds (Xie et al. 2015). Silk fibroin provides a striking example for the formation biomimetic hydroxyapatite. Shepherd and Best (2013) reveal that silk fibroin may be a biocompatible material because it manages the expansion of mineral.

Additionally, zinc ion–hydroxyapatite may increase a lot of biological functions to the nanoparticles, like medicine variation (Velard et al. 2009), bactericide property (Shepherd and Best 2013; Thian et al. 2013) and bone-forming cell response (Webster et al. 2004). The inorganic phosphate precipitates may maintain the mineral section once Zn/(Zn1Ca) reached 15–20 mildew (Ren et al. 2009). The crystal formation of hydroxyapatite is influenced by interaction of sodium alginate and zinc ions with silk fibroin. Culturing of MC3T3-E1 cells onto GO-gel surfaces for differ-

ent cellular activities, bone differentiation and mineralization were civilized. Furthermore, characterization of mineralization was done by alizarin red staining and scanning electron microscopy which finally confirms the deposition of native bone matrix. These studies recommend that the hybrids of GO–gel can have worthy use in surgery of the bone. Scaffolds of polyvinyl alcohol and graphene oxide nanofibrous ready by electrospinning were proved good in bone tissue engineering application (Qi et al. 2013). Cheng et al. (2015) demonstrated formation of the bone by mineralization of hydroxyapatite using polydopamine and reduced graphene compound (RGO-PDA) as a surface.

4 Conclusion

Existing research trends in applications of nanoparticles in different fields of biomedical sciences have been reviewed here. Frequent exciting evidences for nanoparticles to reinforce the faith that use of nanomaterials can develop diagnosis, prevention of disease and treatment which can be of great help to biomedical personals. Although potenial of nano-patterning of nanoparticles in functional medical devices to boost the implant engineering has not yet been fully realized. Nanomedicine research is an exhilarating field which modifies diagnosis and therapeutics study in nearby future. Both clinical and non-clinical pivotal studies must be carried out to assess safety and tolerance of nanomaterials to garner potential commercial application.

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Chapter 12
Nanoparticles and Their Applications in DNA Technology

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Contents

1 Introduction

During the last decade, nanotechnology has produced unique materials with new properties, and it is becoming a promising field in research and development. An exponentially expanding area is bionanotechnology, that is, the combination of nanomaterials and biological molecules. Particularly interesting is gene therapy, which is increasingly used to replace mutated genes with healthy genes, introducing new genetic information into cells to fight against illnesses, such as infectious, neurodegenerative, and cancer diseases, as well as to silence genes that are not expressed properly. The goal of this chapter is to describe some basics of structural and dynamic deoxyribonucleic acid (DNA) nanotechnology to foster interactions among students and scientists from different research areas.

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DNA is a complex structure composed of nucleotides. Its main function is to store the genetic information needed by an organism for its development and survival. Each nucleotide comprises a phosphate group, a 5-carbon sugar group, and a nitrogenous base, which can be either a purine, namely, adenine or guanine, or a pyrimidine, that is, cytosine or thymine (Scofield 2007).

Nanotechnology refers to the manipulation of matter at the nanometric scale, including materials that have at least one dimension in the 1–100 nm range and features such as volume ratio, surface plasmon resonance, quantum confinement, and enhanced mechanical properties, different from bulk materials. By combining nanotechnology and knowledge of the structure and behavior of nucleotides, DNA is automatically taken out of its biologically oriented-only context and treated chemically and structurally through different paths to generate materials that are highly biocompatible and useful for potential biomedical applications (Adams and Barbante 2013; Linko and Dietz 2013).

As will be described, knowing the chemical structure of DNA and its interactions with organic and inorganic materials allows for the design and production of interesting DNA-based nanomaterials through bottom-up and top-down strategies, similar to any other nanostructure. Due to the physicochemical properties and biocompatibility of these nanomaterials, the main applications are oriented toward biomedicine, although applications in electronics have also been considered. Future perspectives, as well as advantages and disadvantages of these materials are discussed at the end of the chapter, justifying extensive research for their creation and use.

2 Structure and Function of DNA

DNA was identified for the first time by the Swiss chemist F. Miescher at the end of the 1860s, when he was studying the protein content of white blood cells. He called it “nuclein” but did not realize its remarkable importance as a biological component. In 1910, the German biochemist A. Kossel won the Nobel Prize for the isolation and description of adenine, cytosine, guanine, thymine, and uracil. Later, the Lithuanian-American biochemist, P. Levene, made two important discoveries: the first one was the determination of the order of the components of a single nucleotide; the second one was the identification of the carbohydrates that belong to DNA and ribonucleic acid (RNA). Moreover, he proposed that DNA was composed equally of adenine, guanine, cytosine, and thymine, but his assumption was disregarded thereafter (Heather and Chain 2016). The Austrian-American biochemist, E. Chargaff, discarded the statement made by Levene, proposing the rules that carry his name: (1) The number of guanine units is equal to the number of cytosine units, and similarly, the number of adenine units equals the number of thymine units; (2) The DNA composition varies depending on the species. These rules already implied that DNA was the carrier of genetic information and not merely a molecule found within cells. The nucleotides appear in pairs of nonarbitrary combinations: adenine

pairs with thymine and guanine with cytosine, by means of hydrogen-bonded pairs of bases at corresponding positions (Watson–Crick base pairing). The order of these nitrogenous bases dictates the gene code or a DNA-encoded set of instructions regarding how to synthesize proteins (Sinden 2012).

The contribution that led to the description of DNA structure and its definition was made by M. Wilkins and R. Franklin. By analyzing X-ray images of DNA, they realized that the two strands that compose the DNA are antiparallel, which implied the DNA's helical structure. Wilkins showed J. D. Watson these results. Subsequently, in 1962, J. D. Watson and F. H. C. Crick won the Nobel Prize for the discovery of the structure of DNA, which was shared with only Wilkins because Franklin had died in 1958 (Olby 2013).

On the other hand, N. C. Seeman is recognized as the founder of DNA nanotechnology, which is also known as nucleic acid nanotechnology. In 1980, he conceived of a three-dimensional (3D) lattice with the ability to entrap molecules of interest, such as proteins. He also proposed that DNA arrays could be used to model assemblies of other molecules that are difficult to handle by conventional procedures. Eleven years later, he and his group reported the synthesis of the first 3D nanoscaled material: a cube made of DNA. Another significant contribution from the Seeman group was the creation of the first DNA nanomachine (Seeman 2003). Many studies have since been dedicated to research related to synthesis procedures, DNA motif analysis, the development of devices, and computational analysis (especially for the prediction of DNA-based structure conformation using programs for molecular and thermodynamic modeling), among others.

It is possible to synthesize complex structures by means of DNA nanotechnology because their assembly relies on the base-pairing concept of nucleic acid molecules (made up of sequences of nucleotides) and DNA sequence motifs. The nanostructure design works such that DNA strands form junctions to create branched structures in the correct arrangement. Additionally, the generation of DNA-based nanomaterials must meet certain desired functionalities for further applications (Ito and Fukusaki 2004). For example, one of the latest and most useful methods to synthesize DNA-based nanostructures is DNA origami, which was reported by P. W. K. Rothemund. It consists of folding single-stranded DNA to create 2D and 3D nanostructures (Rothemund 2006). As explained later in this chapter, the process is accomplished by annealing templates with several DNA strands named "staples."

3 DNA Nanotechnology

DNA nanotechnology refers to the design and synthesis of artificial nucleic acids for applications in biotechnology, biomedicine, and more (Seeman and Sleiman 2017). The structure of DNA itself is in the nanometer domain, with a diameter of approximately 2 nm and a 0.34-nm base-pair separation of its bases. Given that DNA is not a linear molecule, it is necessary for it to be manipulated in order to create nanostructures. So-called motifs, which are defined biologically as short

repetitive patterns that have been assumed to possess biological functions whose task is to indicate sequence-specific binding sites for proteins such as nucleases and transcription factors (TF), are important tools in such a manipulation. Some motifs influence important processes at the ribonucleic acid (RNA) level. RNA is also a nucleic acid assembled as a chain of nucleotides; however, generally, it is a single strand folded onto itself and not a paired double strand like DNA. Furthermore, it differs from DNA by the content of the nitrogenous base uracil, instead of thymine. In nanotechnology, motifs are treated as junctions. They usually possess from 4 to 12 arms along with several helical domains, that is, they are branched constructs. These motifs can be joined as desired by sticky-end cohesion. The joining is mediated by hydrogen or covalent bonding. Other ways to design DNA nanostructures utilize paranemic interactions of double helices, such as some single-stranded knots, Borromean rings, and polyhedral catenanes (Seeman and Belcher 2002).

DNA nanotechnology is subdivided into two subfields: structural and dynamic. The first focuses on the synthesis and description of nucleic acids, as well as on materials which generate static equilibrium assemblies. For the second one, it involves nonstatic complexes under nonequilibrium conditions. In the next sections, aspects of structural and dynamic nanotechnologies will be briefly explained. The classifications of DNA-based nanostructures from several authors will be mentioned as a reference and to emphasize the richness of potential applications of such structures, predominantly in the biomedical area. Another aspect to consider as an advantage is the fact that some of these DNA-based nanostructures already exist in nature. To take advantage of them, it is necessary to exert some chemical or molecular manipulations. Other DNA-based nanostructures are synthetically produced, but still function as biologically generated materials. Combinatorial chemistry, along with molecular biology, is a path that has been taken in DNA nanotechnology because of its uniqueness and proficiency in the synthesis of uncommon molecules. One of the most important methods within combinatorial chemistry is the systematic evolution of ligands by exponential enrichment (SELEX), which has been used to produce synthetic DNA molecules such as aptamers and DNAzymes (Seeman 1998).

Supramolecular chemistry has also been applied to construct nanostructures. This approach is interesting because it integrates DNA along with other materials that are inorganic, organic, and polymeric (Conway and Sleiman 2017). In the following sections, some areas regarding structural and dynamic DNA nanotechnology according to the classification by Connolly et al. (2018) will be described briefly. Topics such as the DNA origami construction technique, i-motifs, and catenanes, which are used for both structural and dynamic DNA-based nanotechnology, will also be discussed.

3.1 Structural DNA Nanotechnology

This branch of DNA nanotechnology has been studied for over 30 years, showing exponential advances since its inception. With structural DNA nanotechnology, building 2D and 3D objects of diverse nanosizes and complexity has been

attained, using the bottom-up approach with the aim of generating static structures (Seeman 2005).

The first DNA objects at the nanoscale were created by N. C. Seeman and his collaborators with the aid of DNA-based scaffolds similar to Holliday junctions, that is, structures that contain four double-stranded arms joined together, which can vary their conformation depending on the sequence of nucleobases closest to the junction and are biologically generated in nature. These static and branched structures led to the design of 3D nano-objects. In 1991, a 3D nanocube, composed of only four single strands resembling a Holliday junction, was built by Seeman and colleagues. Later, another type of branched junction was synthesized, the double crossover molecule, in which two DNA double helices aligned side by side were joined by crossovers. Such configurations can be used to assemble tiles and motifs. Eventually, the triple crossover molecule was designed. Its structure is basically a triple parallel arrangement of double-stranded DNA motifs fastened together and joined through two crossovers amidst each of the double helices. Other structures, such as 2D and 3D lattices (extended), classified as tiles, were also developed; however, control was difficult to accomplish. When the origami technique proposed by Rothemund became popular, it helped somewhat to overcome this inconvenience (Evans and Winfree 2017).

3.1.1 G-quadruplexes and Z-DNA

G-quadruplexes and Z-DNA are non-B forms of DNA. G-quadruplexes (G4) form in nucleic acids through guanine-rich sequences and are helicoidal in shape, enclosing guanine tetrads (G-quartets) that can form from one to four strands. G-quadruplexes are usually stabilized by potassium or sodium cations when they coordinate with the carbonyl groups of guanine. They are polymorphic constructions due to strand orientation, the *syn/anti* glycosidic guanine conformation, and loop connectivity. Biologically, they participate in mechanisms such as DNA replication and transcription and are related to the regulation of gene expression and genome maintenance as well. These constructions have been reported to serve as therapeutic targets in cancer (Kwok and Merrick 2017) and as sensing probes (Ruttkay-Nedecky et al. 2013).

On the other hand, Z-DNA is a left-rolling double-helix structure. This configuration is attained by means of alternating purine and pyrimidine base sequences during DNA transcription. Its biological activity involves affinity to and recognition of molecules such as the poxvirus protein E3L and an ortholog of the protein kinase PKR (Phan et al. 2006).

3.1.2 I-motifs

So-called i-motifs are secondary DNA structures that have four branches that can be generated in cytosine-rich sequences. They consist of two parallel-stranded DNA duplexes which are held in an antiparallel orientation by means of intercalated

cytosine–cytosine base pairs. The folding of i-motifs is pH dependent. Hence, in nanotechnology, i-motif-forming DNA sequences have been used as pH commutators. Originally, it was thought that i-motifs remained unstable under physiological conditions; however, further studies proved that their stability relies greatly on environmental conditions and on their nucleotide sequences. I-motifs are found at sites related to gene regulation, that is, DNA regions that contribute to the activation or silencing of genes, thereby triggering certain molecular mechanisms. It has also been found that they possess key regulatory functions in the genome. Because of their high versatility, static and dynamic DNA structures can be built from them (Dong et al. 2014).

3.1.3 Catenanes

Catenanes are molecules very similar to chains made up by at least two cyclic compounds. In DNA nanotechnology, they are built by the unification of i-motifs with DNA assemblages. Catenanes are generated in a knot shape by topoisomerases to regulate replication and gene expression. Their production for nanotechnology is based on the use of numerous single- or double-stranded templates, within which there are regions of DNA complementarity. Catenanes are interlocked structures.

3.1.4 Aptamers

Aptamers are engineered species of nucleic acids, that is, they are synthesized and selected in a repetitive path in vitro by SELEX. The methodology was reported independently by two research groups, one led by L. Gold and the other by J. Szostak, at the beginning of the 90s. SELEX is the main method for the selection of aptamers, although there are also other methods available. The process was automatized by A. Ellington, who decreased the number of iterations necessary for selection from 6 weeks to only 3 days (Gold 2015). Aptamers have been found to be useful in biomedical applications because of their high specificity and nonimmunogenicity (Sun et al. 2016).

3.1.5 DNAzymes

DNAzymes (catalytic DNA) are DNA molecules (oligonucleotides) that have catalytic properties and are generated through in vitro selection. As aptamers, they cannot be found naturally, but they resemble enzymes. DNAzymes have been used in sensing and biomedicine (Morrison et al. 2018) and in the field of molecular computing systems (Zheng et al. 2019).

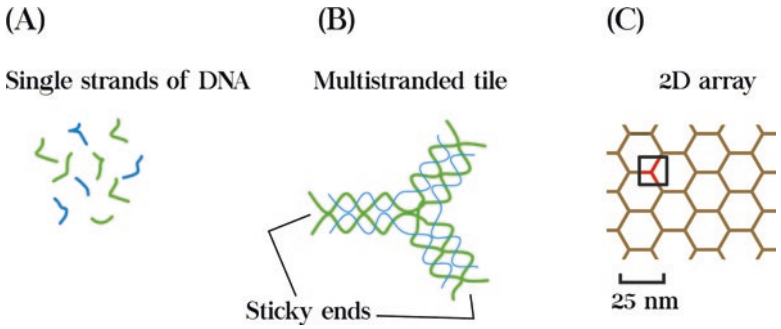

Fig. 12.1 (**a–c**) Scheme showing the formation of a 2D DNA array constructed from single strands of DNA

3.1.6 DNA Origami

Folding single-stranded DNA template molecules by self-assembly into target structures is referred to as DNA origami (Fig. 12.1). This is achieved by annealing templates with DNA strands. Hydrogen bonding formed between adenine–thymine and between cytosine–guanine enable complementary DNA strands to form into a double helix. Normally, the two DNA strands are complementary. Nevertheless, if both strands are only partially complementary, the two strands can accept multiple DNA molecules. As already mentioned, during cell division, DNA can form a four-armed structure known as a Holliday junction. DNA structures containing six strands, stick cubes, branched DNA crystals, and tubes have been produced. Currently, many different shapes can be synthesized and folded (Kuzuya and Komiyama 2010).

3.2 Dynamic DNA Nanotechnology

Dynamic DNA nanotechnology is focused on elements that are independent, can be structured continuously, and work under nonequilibrium dynamics and that have moving parts (animated) plus time-varying performance. It is basically a fusion of self-assembly via programmed hybridization, along with either DNAzyme catalysis or DNA strand displacement reactions. Strand displacement reactions refer to the stepwise substitution of one strand in a double helix with another, invading strand set off by short overhangs of unpaired nucleotides named toeholds. These reactions occur naturally by means of three- or four-way branch migration and originally sparked interest because of their importance in genetic recombination (Simmel et al. 2019). The major drawback of strand displacement and hybridization is that the resulting arrangements may have unwanted leaking reactions that affect the functioning of the synthesized structure. Additionally, even though strand

displacement and hybridization are the main mechanisms responsible for most of the reported structures in the literature, there are other reactions that also yield DNA-based dynamic structures. Research on dynamic DNA nanotechnology is mainly directed toward the synthesis of responsive materials and devices and toward hybridization networks (Zhang and Seelig 2011).

Nonbiologically oriented interest in strand displacement reactions began when, in August 2000, B. Yurke introduced the concept of isothermal strand displacement, which occurs when two DNA strands partially or fully complementary hybridize, provoking a displacement of prehybridized strands for the construction of DNA tweezers. In this structure, the strands that do not get hybridized are practically suspended from the edge of the tweezers. The tweezers open and close by means of fuel strands, which are complementary to the loose ends (Yurke et al. 2000).

3.2.1 DNA Actuators

DNA actuators operate by twisting and untwisting a double helix that is very much alike to a DNA molecule. They have the ability to apply both push and pull forces. Therefore, although designed for cable-driven robotics, these actuators may work as part of other systems (Zampaglione et al. 2019). They are artificial molecular switches. DNA actuators can be subdivided into two groups: DNA switches; and DNA springs, gears and tubes.

3.2.2 DNA Walkers

DNA walkers are synthetic mimics of naturally occurring molecular walkers, such as dynein, myosin and kinesin, in which a nucleic acid can move along a well-designed track assembled partially or entirely from DNA building blocks. DNA walkers also require a DNA track and fuel molecules, or any other form of energy input, to drive the motion. They are characterized fundamentally by their dynamic interactions with the substrate. DNA walkers can be autonomous or nonautonomous (Mason et al. 2018).

3.2.3 DNA Origami Machines

DNA origami machines are dynamic DNA elements that have functional applications in disease diagnostics and treatment. DNA machines are operated by DNA strands and external stimuli to do linear, rotational, and reciprocating movements. Additionally, complicated systems have been created on DNA nanostructures by aligning molecules and molecular machines accurately to resemble biological systems (Endo and Sugiyama 2018).

4 Nanoparticles and DNA

4.1 Nanoparticles Integrated into DNA Structures

Nanoparticles of different kinds have been integrated into DNA structures, such as aptamers and nanocages, to construct complex materials with several functions. Table 12.1 summarizes part of the research work performed on this specific topic.

4.2 Nanoparticles Integrated into or Templated by DNA

Most of the materials mentioned in this section are nanocomposites, which are defined as hybrid materials in which at least one of its components is on the nanometer scale. The objective of combining materials is to promote a synergistic effect.

Table 12.1 Representative publications dealing with DNA structures with integrated nanoparticle systems

DNA structures	Nanoparticles/ nanomaterials	Applications	References
G-quadruplexes	Mesoporous SiO_2 NPs	Locking and unlocking of substrates trapped within the nanoparticles with the aid of G-quadruplexes	Ruttkay-Nedecky et al. (2013)
Hemin/G-quadruplexes	Gold nanoparticles (AuNPs)	Amplified DNA sensing, aptasensing, and detection of Hg^{2+} ions	Pelossof et al. (2011)
Hemin/G-quadruplexes	N-doped graphene/ Au nanoparticles	Ultrasensitive supersandwich-type biosensors for enzyme-free amplified microRNA detection	Wang et al. (2018a)
Aptamer	Thiolated DNA-functionalized AuNPs	Colorimetric sensing of adenosine and cocaine	Liu and Lu (2005)
Aptamer	AuNPs	Electrochemical detection of cortisol	Sanghavi et al. (2016)
Aptamer	Magnetic nanoparticles (MNPs)	Cancer cell targeting	Grobmyer and Moudgil (2010)
DNAzyme	AuNPs	Detection of thrombin through chemiluminescence	Wang et al. (2018b)
DNAzyme	AuNPs	Amplified detection of DNA or telomerase activity	Niazov et al. (2004)
DNA origami cage	AuNPs	Proposed as an artificial structural platform for engineering novel bio-inspired, biomimetic, and biokleptic materials	Zhao et al. (2011)
Actuator	AuNPs coated with biotinylated DNAs	Monitoring of nanoscale movements induced by an electric field	Tapio et al. (2018)
DNA walker	Quantum dots (QDs)	Protocol for assembly	Ke and Wang (2017)

The intrinsic affinity of DNA molecules toward metallic ions aids the formation of metallic nanostructures. DNA template-induced synthesis of nanoparticles has been the subject of extensive study. It will be described because the properties of the materials yielded from DNA templates, nanoclusters, vary according to the base materials used for the particles, such as, for instance, silver, gold, palladium, and carbon. DNA can also be conjugated to nanoparticle surfaces. Metal-based and polymer-based DNA nanoparticles, along with their applications, will be discussed as well.

4.2.1 Gold Nanoparticles

Gold nanoparticles (AuNPs) are among the most studied nanomaterials that have been coupled to DNA, mainly for applications such as sensing. It is interesting to note the wide range of nanostructures that can be produced with gold, from simple spherical nanoparticles up to hybrid and more complex systems for several applications.

Deka and colleagues (2017) exploited the optical properties of the DNA-AuNP system as a colorimetric assay for the measurement of helicase activity. Helicases are vital enzymes for many organisms, and their main task is the unpackaging of DNA. As a first step, the authors synthesized two batches of 20-nm nanoparticles functionalized by DNA (self-assembled monolayer). The difference between the batches was the oligonucleotide sequences used for functionalization. Then, they prepared a substrate by mixing the batches with a duplex to promote complementation of the DNA strands of each batch. The human RecQ4 helicase was used for the trials and prepared in a solution buffer. The buffer was added to the gold nanoparticles with adenosine triphosphate (ATP), varying the concentration of the helicase enzyme in the nanomolar range. Once the enzyme interacted with the nanoparticles and the ATP, there was a color change in the solution which was monitored by means of UV-Vis spectroscopy. This was due to the unwrapping of DNA by RecQ4, breaking the substrate into smaller parts, and releasing the nanoparticles, which was proportional to the enzyme concentration. The highest unwrapping rate was found at the highest RecQ4 concentrations: 100 nM and 150 nM.

Chan et al. (2018) used DNA-functionalized gold nanoparticles to enhance messenger ribonucleic acid (mRNA) translation. Gold nanoparticles were prepared by the citrate method, and later, selected DNA oligomers with thiol ends were attached to the nanoparticles as a conjugation step. Due to their specificity, these selected oligomers were used to assess the dependence of translation enhancement on them and insulin mRNA was used as a model molecule. The synthesized nanoparticles were added to HeLa (cervical cancer) cells lysates, enhancing insulin synthesis by 2.18-fold with just the presence of the mRNA (Chan et al. 2018).

The synthesis and usage of DNA-functionalized gold nanoparticles are not only limited to enzyme activity presence and monitoring but also include the detection of metal ions (Lee et al. 2007), cellular uptake studies (Wong and Wright 2016), protein discrimination through fluorescence (Sun et al. 2015), bacteria detection

Table 12.2 Representative publications dealing with gold nanostructures generated by the DNA-templating technique

Gold nanostructures	DNA templates	Applications	References
Nanowires	DNA origami mold	Potential fabrication of structures with programmable shapes	Bayrak et al. (2018)
Branch-shaped	X-shaped and Y-shaped DNA	Photothermic cancer cell treatment	Song et al. (2017)
Bowtie nanoantennas	DNA origami	Single molecule surface-enhanced Raman spectroscopy	Zhan et al. (2018)
Nanorods	DNA origami clamps	Site-specific surface functionalization	Shen et al. (2016)
Gold nanorod complex	DNA origami	Cancer theranostics	Jiang et al. (2015)
Circles and triangles	DNA origami	Nanoelectronics and plasmonics	Ruiz et al. (2017)

(Arunrut et al. 2016), and the entrapment of the nanoparticles within nanocages in order to be used as delivery and release agents (Chandrasekaran and Levchenko 2016).

DNA can be used as a template for nanoparticle formation to produce AuNPs, as well as quantum dots, and metal oxide nanoparticles, among other structures. The usual DNA templates are short strands with specific sequences, for instance, scaffolds. If the sequence and structure of the DNA template can be tuned, nanostructures with different sizes and from different materials can be generated.

Moreover, DNA–gold hybrid nanomaterials with more complexity have been developed. An example is a multitask platform in which aptamer MUC-1 functionalized-triangle DNA origami was loaded with doxorubicin (Dox) and was also able to transport gold nanorods to suppress the growth of resistant breast cancer cells (Song et al. 2017). Other gold nanostructures that can be created through DNA templates are listed in Table 12.2.

4.2.2 Silver Nanoparticles

Silver nanoparticles (AgNPs) possess much better plasmonic properties than gold nanoparticles, namely, they have higher extinction coefficients; however, studies of AgNPs in combination with DNA are limited. This is attributed to the low bond energy of Ag-S compared to that of Au-S, as well as to their oxidation and aggregation trends. Among those few studies, which are mainly related to fluorescence exploitation, the following one is noteworthy: Divsar et al. (2015) developed aptamer-conjugated silver nanoparticles for the detection of Arsenic (As (III)) ions in solution. The detection mechanism rests on the formation of As(III)–aptamer–silver nanoparticle (Apt-SNP) complexes, which causes a decrement in the absorbance of the conjugated silver nanoparticles. A three-factor, central composite design optimization method, in conjunction with response surface methodology, was used for the maximization of the efficiency of arsenic detection. The nanoparticles were

synthesized using sodium borohydride, and then, polyvinylpyrrolidone was added to the solution; the solution was then stored in the dark and incubated for 18 h with an aptamer solution to promote its attachment through covalent bonding.

In other research works on DNA and silver nanoparticles, which do not involve DNA conjugation to the nanoparticle surface, the template-directed method has been used to create nanoclusters that exhibit bright fluorescence, tunable emission and improved stability by just varying the sequence of the DNA template. These structures mainly take advantage of the generated fluorescence which can be enhanced by the adjustment of complementary DNA for detection purposes. Zhou et al. (2017) developed a platform consisting of DNA-template silver nanoclusters, whose emission could be tuned from yellow to red. This was achieved by hybridization of the nonfluorescent silver nanocluster with A_{20}-C55 complementary DNA (T_{20}) to enhance the yellow color signal and with A_{20}-C55 that shows yellow and red signals of equal intensity in the presence of Mg^{+2} ions. It was reported that the fluorescence could also be reverted by just separating two silver nanoclusters by means of the strand displacement reaction. Other silver fluorescent nanoclusters have been developed to detect pathogenic bacteria such as *Escherichia coli* (*E. coli*). This system was developed as DNA–silver nanoclusters (AgNCs) integrated with an MDA (MNP-DNAzyme-AChE) complex (MNP stands for magnetic nanoparticle and AChE for acetylcholinesterase). Its principle is based on the fact that MNPs act as separated elements, DNAzyme as the bacteria recognition agent, and AChE as the enzyme. After the separation induced by the MNPs, the released AChE is transmitted to the DNA-AgNCs to induce the hydrolysis of acetylthiocholine (ATCh), thereby enhancing the fluorescence of the nanoclusters (Zhang et al. 2013). DNA-AgNCs have also been used for microRNA location (Zhang et al. 2018) and the detection of dopamine (Del Bonis-O'Donnell et al. 2018) and thiol compounds (Huang et al. 2011). In some other cases, the published research has only been focused on the synthesis procedure and mechanisms of silver nanocluster formation (Petty et al. 2004).

4.2.3 Copper, Platinum, and Palladium Nanoparticles

Copper is an element that is widely used in electricity and the production of integrated circuits. Similar to silver, it is not a widely studied material in DNA nanotechnology because of its tendency to cleave DNA molecules. Nonetheless, Cu nanowires can be manufactured from DNA templates, and they are good potential candidates for use in the integrated circuit production field. Double-stranded DNA solutions have been used to functionalize nanoparticles from Cu $(NO_3)_2$ metal precursors. In this process, the positively charged Cu ions associate with the negatively charged DNA phosphate groups, and then, ascorbic acid is added to reduce copper, where it simultaneously forms a coat around the DNA, resulting in the formation of nanowires 3 nm in height (Monson and Woolley 2003).

Copper nanoblocks have been synthesized on different kinds of RNA (mRNA (T1E4), miRNA (miR-107), and lncRNA (SChLAP1) biomarkers) for simultaneous detection of multiple RNA biomarkers related to prostate cancer. This method

involves electrochemical RNA detection. The isolated RNA targets used in the study are adsorbed onto gold electrode surfaces by means of nucleic-acid-based-gold affinity interactions, and then a catalyzed H_2O_2 reduction is induced, allowing for the generation of an electrochemical current that can be measured by amperometry, which reveals the amount of the RNA target. This application has a very interesting perspective because it has an outstanding possibility to be transferred to the clinical domain (Koo et al. 2018).

There are also studies related to DNA-templated Cu nanoclusters for hepatitis B virus detection through a colorimetric approach. This method was first used with DNA as the probe molecule. Good outcomes were achieved mainly because no equipment was necessary to detect the target molecule and because this method could be carried out with only the naked eye, which resulted in an inexpensive and practical setup (Mao et al. 2016).

Platinum nanoparticles (PtNPs) have been functionalized with DNA by the exchange of labile surface ligands with thiol-modified DNA, in a similar fashion to that of DNA functionalization of AuNPs. Nanomaterials that mimic enzymes are referred to as nanozymes. In a publication by Fu et al. (2014), G-C-rich oligonucleotides (AG22 (5′-A(G3T2A)3G33′) and RET2 (5′-GC5(GC4)3T-3′)) were chosen as templates to synthesize peroxidase-mimicking Pt nanozymes with a size distribution of 1.7–2.9 nm and with high activity. The most efficient, synthesized Pt nanozyme was 66% metallic Pt_0 stabilized by the i-motif RET2, with an average diameter of 2.9 nm. The goal of this research was to basically prove the ease of modulation of physicochemical properties by programming the DNA sequences used.

Palladium nanoparticles (PdNPs) possess good catalytic activities and electrochemical properties. Their potential applications are under study. Some reported studies on DNA/PdNPs are limited to their catalytic activity and their relation to certain types of reactions that involve catalysis under certain conditions. One of these systems at the nanoscale to exploit catalysis uses a Pd/DNA catalyst that was shown to have high activity for the selective hydrogenation of carbon–carbon triple bonds, carbon–carbon double bonds, and nitro groups, as well as for a Suzuki–Miyaura coupling reaction, which involves Pd. The motivation behind this study was triggered by the fact that salmon testes contain DNA that could be processed further instead of being discarded as waste. The Suzuki–Miyaura coupling reaction, as well as hydrogenation, facilitates this processing (Itoh et al. 2012).

Another example is a catalyst composed of palladium nanoparticles supported on DNA composed of Pd(II) and Pd(IV) species in their oxide form, which aids in the copper- and ligand-free Sonogashira–Hagihara coupling of aryl iodides with terminal aromatic and aliphatic alkynes. The most important advantages reported were that the catalyst could be easily recovered and reused in five cycles, thereby showing better performance than commercial palladium catalysts. Additionally, such a system achieved 54–86% isolated yields using low catalyst loadings (0.5 mol) under mild conditions (65 °C) in methanol without air exclusion (Camacho et al. 2017). Additional studies on these DNA-based metal nanostructures are mostly related to their construction and characterization but not on a specific application (Song et al. 2015).

4.2.4 Magnetic Nanoparticles

Magnetic nanoparticles can be manipulated using magnetic fields. They consist of two components: a magnetic material (iron, nickel, or cobalt) and a chemical component that has functionality. These nanoparticles have high field irreversibility, a high saturation field, superparamagnetism, extra anisotropy contributions, and shifted loops after field cooling. All these properties arise from narrow and finite-size effects and surface effects that dominate the magnetic behavior of individual nanoparticles (Akbarzadeh et al. 2012). The combination of MNPs and DNA gives rise to nanocomposites that are of two types: DNA on MNPs and NPs on DNA. Their applications are mainly found in nanoelectronic devices, in vivo and in vitro biomedical studies, magnetosensitive biosensors, drug delivery systems for therapy, and magnetic fluorescent nanocomposites (Pershina et al. 2014).

The identification of bacteria in urine samples via rolling circle amplification, MNPs, and a simple reader based on low-cost optical components was proposed by Mezger et al. (2015). *E. coli* was successfully detected in clinical samples with no false negatives and a total assay time, including sample preparation, amplification, and detection, under 4 h; this result is very promising in terms of practicality. The synthesized MNPs were coated with universal readout probes that matched generic sequences on the backbone of the padlock in an agglutination assay format. Fluorophore-labeled Fe_3O_4 NPs attached to single-stranded DNA were synthesized for the detection of pyrophosphate anion ($P_2O_7^{4-}$, PPi) in the synovial fluid. The concentration of proton pump inhibitors (PPi) was found to be in the $2.0 \times 10^{-7} - 4 \times 10^{-6}$ M range, with a detection limit of 76 nM. These results could be valuable for the diagnosis and therapy of arthritic diseases in the future (Tong et al. 2015).

4.2.5 Quantum Dots

Quantum dots (QDs) are artificial semiconductor crystalline nanoparticles that have applications in composites, solar cells, and fluorescent biological labels. Their sizes are between 2 and 10 nm. QDs are generally constructed from elements of group II (e.g., Zn, Cd), group VI (e.g., Se, S), groups III and V, and groups IV and VI of the periodic table, and they possess unique physical and optical properties. Their functionalization using DNA has been under study providing a good platform for applications, such as gene expression quantification and imaging, single-molecule imaging, live tracking of proteins, and self-assembly (Banerjee et al. 2016).

Mirkin (2000) published an article entitled "Programmed assembly of DNA functionalized quantum dots," in which they basically reported the first successful modification of semiconductor nanoparticles with single-stranded DNA, the generation of DNA-linked QD assemblies, and a preliminary account of their optical properties. Such an investigation was a milestone for the QDs/DNA combination.

Cadmium-free DNA-functionalized Mn-doped ZnS (DNA-ZnS:Mn^{2+}) QDs have also been studied. These QDs were found to have excellent photo-stability with the

Table 12.3 Representative publications dealing with DNA-quantum dot composite systems

System	Application	References
DNA-conjugated QDs assembled on photoactive thin films	Optoelectronics	Noh et al. (2014)
QDs conjugated to single-stranded DNA	Ratiometric detection of unlabeled DNA	Page et al. (2016)
QDs-DNA hydrogel	Delivery of Dox to cancer cells, increasing the potency of the drug	Zhang et al. (2017)
DNA nanocage-quantum dots complex	Biosensing	Wang et al. (2016)

help of polyacrylic acid (PAA) and DNA. A Förster resonance energy transfer (FRET) model utilizing these QDs and WS_2 nanosheets as energy donor-acceptor pairs, which was applied to protein detection through the terminal protection of small molecule-linked DNA, with positive outcomes, was reported by Zhang et al. (2017). Table 12.3 shows information on DNA-based quantum dots to enhance their potential as tools, especially for biomedical applications.

4.2.6 Other DNA-Based Nanosystems

The aforementioned studies dealt with nanomaterials comprising DNA and a nanostructure of only one type, for example, DNA–gold nanostructures and DNA–silver nanostructures, but there is also research on the synthesis of nanoparticles that involve DNA to generate more complex nanocomposite structures that might combine gold–silver nanoparticles, graphene-AgNCs, etc., with DNA-only-based structures such as cages, origami, and aptamers. Additionally, other variations of DNA-directed synthesis have been considered. For example, DNA templates have been used to synthesize combined metals or other combinations of nanomaterials. Table 12.4 lists some of these studies for further reference.

4.2.7 Biodegradable and Metal DNA Nanoparticles for Transfection

Biodegradable polymer-based nanoparticles have been developed to be functionalized by or to encapsulate DNA in the form of plasmids to genetically transform cells. Plasmids are loop-shaped forms of DNA that are found in bacteria and in some eukaryote cells. When used for cloning, transferring, and manipulating genes, they are called vectors. These vectors are incorporated as functionalizing agents or as encapsulated components within nanoparticles. Biodegradable polymers have been used for this purpose because they protect the plasmid. After entering into the cells, they are easily degraded, releasing the plasmid within the cell so that it can be replicated. Because of their biocompatibility, gold nanoparticles have also been used to transfect cells. The most common polymers used in the synthesis of this kind of nanoparticles are chitosan and sodium alginate. The procedure to synthesize

Table 12.4 Representative publications dealing with DNA sequence-based nanocomposites

Systems	DNA type	Applications	References
Ag/Au NCs	5′-CCCTTAATCCCC-3′ DNA sequence	Detection of sulfide ions	Chen et al. (2011)
Cu/Ag NCs	5′-CCCTTAATCCCC-3′ DNA sequence	Detection of copper ions	Su et al. (2010)
AgNCs-Graphene oxide	Oligonucleotide DNA	Sensitive fluorescence-based detection of multiple nucleic acid targets	Tao et al. (2012)
Ag/Pt NCs	5′-CCCCCTAACTCCCCC-3′ DNA sequence	Vascular endothelial growth factor (VEGF) detection	Fu et al. (2016)
Fe/Pt NPs	(5′-TAATACGACTCACTATAGGGAGACCACAACGGTTTCC-3′) and S2 (3-ATTATGCTGAGTGATATCCCTCTGGTGTTGCCAAAGG-5) ssDNA self-assembly	Not specified	Srivastava et al. (2007)
DNA-modified Fe_3O_4@Au magnetic nanoparticles	DNA 1 was thiolated with a –(CH2)6– spacer at the 5′-end. DNA 2 and DNA 3 were labeled with Fc and MB at the 5′-end, respectively	Detection of heavy metal ions	Miao et al. (2017)

the nanoparticles is usually the ionic gelation method. DNA interacts with the polymer by means of electrostatic interactions. Once the nanoparticles have been synthesized, they are mixed with a solution of the microorganisms or cells to be incubated and transformed. For comparison purposes, a control is usually performed with a chemical transfection reagent and the cells or microorganisms to be transformed. Chitosan nanoparticles have been thiolated for the transfection of $CaCo_2$ cells (Martien et al. 2008), and sodium alginate nanoparticles have been combined with chitosan to provide better transfection efficiencies in NIH 3T3 cells.

5 Conclusion and Future Perspective

DNA has proved to be an excellent molecule for use in nanotechnology in many of its forms because it is highly versatile and can be programmed and manipulated at the nanoscale. The synergy between nanoparticles and DNA nanotechnology is a very useful interaction that takes advantage of DNA programmability and its feasibility as a conjugating or templating agent, providing nanoparticles with improved properties in comparison with other approaches. Disadvantages of DNA nanotechnology include the high costs involved in the production of synthetic DNA, low yields, and high sensitivity of the generated structures, especially to temperature variations and nucleases. Nonetheless, some solutions to these limitations have been proposed (Tørring and Gothelf 2013).

DNA is a promising tool for the synthesis of materials in the form of oligonucleotides, single-stranded or double-stranded forms, or plasmids, with impressive traits that can be used in areas such as biomedical applications and nanoelectronics.

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Chapter 13
Nanoparticles on Photosynthesis of Plants: Effects and Role

Kasturi Poddar, Debapriya Sarkar, and Angana Sarkar

Contents

1 Introduction

Nanoparticles are one of the emerging interests in recent times due to their variable applications in different sectors of society, which include agricultural industries, chemical industries, electronic industries, oil industries, consumer goods industries, biotechnology industries along with their use in energy production. Nanoparticles (NPs) are distinct entities by virtue of their morphology, for instance, nanoscopic size ranging from 1 to 100 nm, crystalline or amorphous shape, natural or anthropogenic origin, and chemical composition based on metal, carbon, composites, or dendrimers. With time, nanoparticles have acquired extensive use in various biological processes along with the other physical and chemical processes aiding their potential use in the environment as well as human health. The distinct small size of the nanoparticles makes them favorable for penetration through cells to reach specific target locations, thereby increasing interactions with cellular components. The high volume to the surface area ratio of these structures enables them for faster adsorption and permitting more target-specific delivery of compounds (Khan et al. 2017). These impart specific properties to the nanoparticles and make the intermediary components between bulk-sized parent components and atomic-sized molecular elements.

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Further classification of nanoparticles based on their morphology differentiates between low or high aspect ratio, isometric or asymmetrical in uniformity, and composed of single material or composites (Tighe-Neira et al. 2018). Significant characteristics including a hike in the catalytic and photocatalytic activity are observed for (ZnO)NPs, (TiO_2)NPs, and (CeO_2)NPs. Localized adsorption of different materials on the surface of other metal (gold, silver, and platinum) nanoparticles was also observed. Also, higher surface reactivity of these particles escalates the surface chemistry modification and, thus, makes it appropriate for use in antimicrobials (Vollath 2013). Based on the modifying property of nanoparticles, they have been accepted industrially for technology, medicine, and environment (Corujeira et al. 2017; Simon et al. 2018).

Industrial and commercial use of nanoparticles has increased the release and accumulation of nanoparticles in our soil and water bodies even to the groundwater level. Generally, the nanoparticle used in the different field tends to accumulate in the dumping region and eventually get washed out to other neighboring regions. Besides this, nanoparticles are being involved in a wide range of chemical, physical, and biological processes, and they tend to bioaccumulate and biomagnify and are thus found in escalated concentrations in the modern-day food chain (Fig. 13.1) (Sarkar et al. 2019). Nanoparticles like copper oxide have wide use in electronic sensor devices, which include glucose sensors, pH sensors, and amino acid detectors

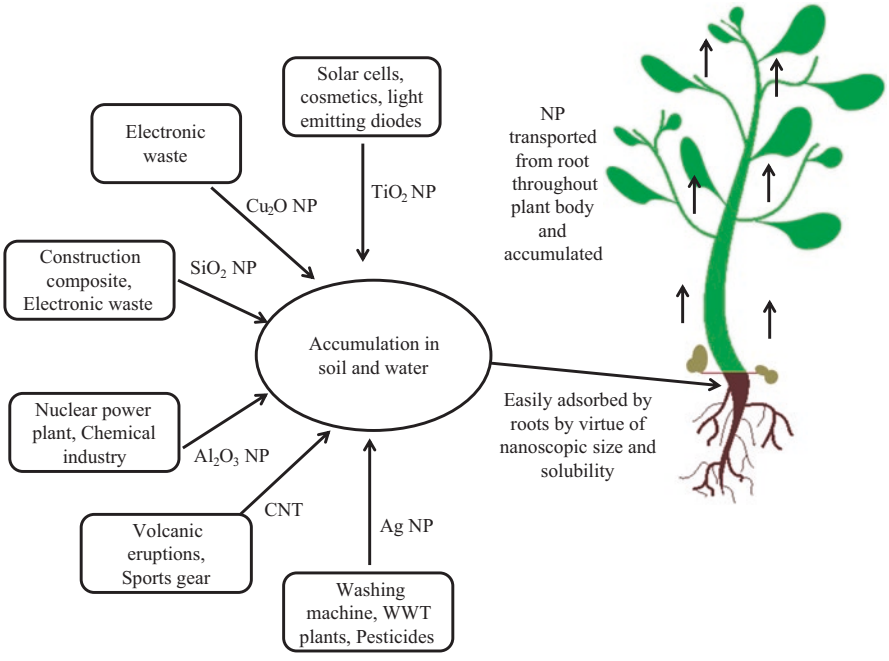

Fig. 13.1 Increase of nanoparticle concentration in plants due to bioaccumulation and biomagnification or different nanoparticles from various natural and anthropogenic sources

(Ibupoto et al. 2013). They are also widely found in various electronic goods, such as mobiles, calculators, computers, and thermostable devices. Hence, the sources for copper oxide nanoparticles contamination are electronic dumps. Silver oxide nanoparticles possess antimicrobial benefits and thus are often utilized in different cosmetic compounds, textile industries, dishwashers, washing machine, pesticides, wastewater treatment plants, etc., and nanoparticles from these get eventually washed out, thus contaminating the soil and aquatic environment (Aziz et al. 2016; Joshi et al. 2018). Titanium oxide from solar cells and light-emitting diodes are susceptible to wash out from the user devices and get cumulated in the environment producing nanotoxicity pollution (Environmental Working Group 2017). Aluminum oxide or alumina nanoparticles are widely utilized in different industrial downstream processes, especially in air filters, and also utilized as a drying agent or desiccator. Wastewaters generated out from nuclear power plants are also found to contain alumina nanoparticles (Sadiq et al. 2011). Other nanoparticles like silicon dioxide nanoparticles are found in contamination sources like batteries, semiconductor devices, nanochips, and electronic wastes; for carbon nanotubes, the sources are modern-day sports gear like baseball bat, ice hockey stick, or different industrial structures like arrow bars, wing turbine, or forks. Forest fires and volcanic eruptions are also considered as natural sources for carbon nanotubes contamination sources (Murr et al. 2004; Sarkar et al. 2019). The major problem concerned with nanopollution and nano-contamination is that the mean of identification and localization of the nano-contaminants are very restricted if present at all. Bioaccumulation and biomagnification are the only ways for detection of such nano-contaminants. Hence, the study of the effects of these contaminants is very essential to monitor the level of contamination. This chapter explains the effects of different nanoparticles on plant photosynthesis.

2 Nanoparticles and Its Effects on Plant System

A variety of metallic nanoparticles are already existing in the environment in association with several plant species; however, their effects on plant's growth and phenotypic parameters may vary among species. The effect of the nanoparticles on plants mostly varies due to growth conditions, growth stages, exposure time, and applied dosage (Rizwan et al. 2019). Nanoparticles help in seed germination of plants by activating the aquaporin controlling genes, thereby improving the water uptake along with the penetration of nanoparticles. This regulates the cell cycle and helps in improved seed germination in plants (Khan et al. 2017). Along with the amount of water uptake, present of sufficient food as enzymes like amylase and protease promotes survival of the plant. TiO_2 nanoparticles at low concentration increase the activity of amylase and protease enzymes (Laware and Raskar 2014). Along with several negative impacts on plants, nanoparticles also induce beneficial effects to their associated plants in forms of abiotic stress resistance by antioxidant

compounds, positive biostimulation, higher biomass production, and end products with higher quality standards (Tripathi et al. 2017; Apodaca et al. 2018). Titanium dioxide (TiO_2) nanoparticles also produce significant beneficial effects on plants like *Arabidopsis thaliana, Lactuca sativa, Avena sativa, Linum usitatissimum,* and cabbage. It improves the germination of seeds, extends root length, and enhances the growth of seedling plants (Aghdam et al. 2016; Andersen et al. 2016; Szymanska et al. 2016). Further increased crop yield and biomass were observed for plants like tomato, wheat, and corn (Morteza et al. 2013; Rafique et al. 2015; Raliya et al. 2015).

The abiotic stress tolerating various antioxidant targets the reactive oxygen species (ROS) released by different cell organelles on exposure to oxidative stress caused by abiotic conditions like high salinity, drought, unfavorable temperature, heavy metal concentration, floods, or ultraviolet radiation. Moreover, the antioxidant compounds also provide a defense to the plants in such stress by stimulating the antioxidant activities and accumulating nutrients, osmolytes, and amino acids to plant system to attain the defensive action against the damage. Nanoparticles associated with soil minerals like analcite [$(AlSiO_2O_6)$-H_2O] help induce drought resistivity to wheat and corn plants by facilitating their antioxidative action with the production of photosynthetic pigments (Zaimenko et al. 2014). In another study, it was also observed that foliar usage of iron nanoparticles induced stomatal closure in the safflower plant to combat the water scarcity in drought conditions (Zareii et al. 2014). However, it was also observed that noncompatible or high concentration of nanoparticles may also block the root cells by adhering to them and prevent water and nutrient adsorption (Martínez-Fernández et al. 2016). Nanoparticles associated with plants at the hypothermic condition or chilling stress influence the upregulation of *MeAPX2* and *MeCu/ZnSOD* genes that elevate the activity of the antioxidant system components like dehydroascorbate reductase, monodehydroascorbate reductase, and glutathione reductase that combat the generated ROS within the plant cell. The report suggested that application of nanoparticles like SiO_2 NPs and, in some cases, TiO_2 NPs assists this stress tolerance mechanism in plants (Haghighi et al. 2014). Under heat stress, plant cells tend to produce molecular chaperone named heat shock proteins (HSPs), which provide plant cells thermotolerance. It was evident in studies that carbon nanotubes help to maintain the sublime expression of these (Khodakovskaya et al. 2011). Other nanoparticles like cerium oxide (CeO_2) and TiO_2 NPs also have some reports with benefit for plant survival in heat stress. However, the contribution of these nanoparticles in photosynthesis is still a concern for further investigations (Zhao et al. 2012; Qi et al. 2013). Other forms of abiotic stress like salinity or heavy metal contamination induce different plant defense mechanism which targets to compensate or nullify the generated ROS within the cell. Silicon oxide and titanium oxide in some cases have reports to help these defense mechanisms by either reducing the accumulation of heavy metal or maintaining membrane integrity to preserve the desired salt concentration within the cell (Ali et al. 2019; de Sousa et al. 2019; Rizwan et al. 2019; Singh and Lee 2016).

3 Influence of Nanoparticles on Photosynthesis

Since photosynthesis is an exclusive approach to harness energy for the plants, the involvement of the nanoparticles and their effects on photosynthesis activity are a concern for recent studies. Some of the beneficial effects of nanoparticles noted of plant development and photosynthesis had been discussed for some plant species (da Costa and Sharma 2016; Sarmast and Salehi 2016; Zarate-Cruz et al. 2016; Cao et al. 2018). The detailed interaction of the nanoparticles with the molecular and ultrastructural components of plant photosynthetic system needs to be established to analyze the rate of the energy transformation occurring in the plants (Hossain et al. 2015; Du et al. 2016; Panpatte et al. 2016; Sarmast and Salehi 2016; Tripathi et al. 2017). Hence, nanoparticle interaction with plants and their impacts on plant physiological processes along with biological modifications of the photosynthetic system makes it the center of analysis and the interest of study.

Photosynthesis relies more on the structural configuration of the participating organelle that is responsible for maintaining the gaseous concentrations within the apparatus and controlling carbon dioxide circulation to the sites of carboxylation (Mediavilla et al. 2001). Other factors that influence the efficiency of the photosynthesis include the structural integrity of chloroplasts and mesophyll cells, adequate grana development, carbon dioxide aggregation, activity of photosynthetic enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo), and adequate presence of pigments that aids photosynthesis like chlorophyll a and chlorophyll b along with the regulatory proteins of the thylakoids (Wang et al. 2014; Sáez et al. 2017). Hence, positive impacts produced on the photosynthesis efficiency with the alteration of the factors responsible for photosynthesis yield an option for crop improvement (Foyer et al. 2017).

Light energy absorbed during photosynthesis is essentially converted into chemical energy, yielding different elements of the photosynthetic reaction. Nanoparticles can impose both positive and negative impacts on photosynthesis. It influences the light-harvesting complex of plants by enhancing the reaction, as well as inhibits the electron transport system, and alters the activity of RuBisCo, carbonic anhydrase, or phosphoenolpyruvate carboxylase (PEP) enzymes, thus preventing the metabolism pathway (Kataria et al. 2019). Scientists are currently working to produce higher crop yield by improving the photosynthetic efficiency of plants using embedded SWCNTs in their chloroplasts. SWCNTs enhanced the transport rate of electron and improved the biochemical sensing of signaling molecules like nitric oxide (Giraldo et al. 2014). Since the nanoparticles affect the functionality of the photosynthetic elements, thorough research with time is required to evaluate the impacts caused by the nanoparticles on the final products of the photosynthetic process. Utilization of a conjugate of silicon compound with photosystem II provided a stable photosynthetic reaction for oxygen development, which enhanced the activity of photosynthetic enzymes and pigments. The conjugate could also act as photosensors in artificial photosynthesis (Xie et al. 2012; Siddiqui et al. 2014, 2015).

4 Different Nanoparticles Affecting Plant Photosynthesis

Metallic nanoparticles tend to inhibit photosynthesis by the generation of oxidative free radicals or reactive oxygen species (ROS) that oxidizes the various photosynthetic components. The triggered responses caused by nanoparticles vary among different plant species as discussed in Table 13.1.

Table 13.1 Nanoparticles and their effects on photosynthesis

S. no.	Nanoparticle	Target site of photosynthesis	Effects	Remarks	References
1.	Zinc oxide NP	Chlorophyll biosynthesis genes like chlorophyll synthetase (Chlg), Copper response defect1 (Crd1), Mg chelatase Subunit D(Chld), chlorophyll A oxygenase (Cao), Mg-protoporphyrin IX methyl transferase (Chlm) Photosystem (PS) I structure gene like photosystem I subunit D2 (Psad2), PS I subunit N (Psan), PS I subunit E2 (Psae2), PS I subunit K (Psak)	Inhibits biosynthesis of chlorophyll and also hampers the photosystem I; hence, reduces the photosynthetic ability of the plant	Harmful	Wang et al. (2016)
2.	Copper (II) oxide NP	Chloroplast	Decreases thylakoids number in granum of chloroplasts. Inhibits the expressions of photosystem I proteins and completely demolishes photosystem II at higher concentration	Harmful	da Costa and Sharma (2016)
3.	Carbon nanodrops	Photosystem	Improve electron transfer in photosystem	Helpful	Wang et al. (2018)
4.	Super paramagnetic iron oxide NP (Fe_3O_4, $Co_{0.2}Zn_{0.8}Fe_2O_4$, $Co_{0.5}Zn_{0.5}Fe_2O_4$)	Chlorophyll synthesis, photosystem II	Produces loads of reactive oxidative species which causes destruction of chlorophyll and other building parts of photosystem II causing shut down of whole photosynthesis process to huge extent	Harmful	Barhoumi et al. (2015)

(continued)

Table 13.1 (continued)

S. no.	Nanoparticle	Target site of photosynthesis	Effects	Remarks	References
5.	Titanium oxide NP	Chlorophyll a content and photosystem II	Decreases chlorophyll a content and decreases the efficiency of photosystem II. No effect on RuBisCo, and total soluble sugar (TSS) was reported though	Harmful	Dias et al. (2019)
6.	Silver NP	Chlorophyll content	Significant oxidation of chlorophyll as well as components of photosystem. It also inhibits plant growth	Harmful	Li et al. (2018)
7.	Gold NP	Photosystem II	Au NP enables the reabsorption of photoemission from photosystem II, hence enhances the photosynthesis efficiency	Helpful	Torres et al. (2018)
8.	Silicon dioxide NP	Detoxification, oxidative stress relief	SiO_2 NP helps plants to grow in deferent metallic stress by reducing the uptake of the metallic contaminants as well as by protecting the antioxidant system of plant	Helpful	de Sousa et al. (2019) Rizwan et al. (2019) Ali et al. (2019)
9.	Aluminum oxide NP	Chlorophyll and other photosynthetic pigments and photosystem components	Al_2O_3 NP creates oxidative stress in plants and causes damage on photosynthetic pigment systems	Harmful	Yanık and Vardar (2018)
10.	Single-walled carbon nanotube	Electron transport in photosystem and antioxidant system of chloroplast	It enhances the electron transport in chloroplast, enhances photoabsorption, and enhances the antioxidant system of chloroplast	Helpful	Giraldo et al. (2014)

4.1 Metallic Nanoparticle

The impacts caused by superparamagnetic iron nanoparticles (SPIN) (Fe_3O_4, $Co_{0.2}Zn_{0.8}Fe_2O_4$, $Co_{0.5}Zn_{0.5}Fe_2O_4$) were reported in a recent study on *Lemna gibba* plant model. The net chlorophyll content was stated to have reduced significantly in SPIN-supplemented plant specimens. A significant reduction was also observed in the efficacy of photosystem II due to an elevated amount of ROS present in the SPIN-treated viable cells. Due to the disparity produced in the antioxidant system, further oxidation of components comprising the photosynthetic complex, was observed, thus causing a decline in the photosynthetic yield in SPIN-treated plant specimen (Barhoumi et al. 2015).

Titanium dioxide (TiO_2) nanoparticles induce many positive effects on plants by increasing the chlorophyll content in plants like tomato and oilseed (Raliya et al. 2015; Li et al. 2015). It was also found to exhibit the higher enzymatic activity of RuBisCo in *Arabidopsis thaliana, Ocimum basilicum*, and *Spinacia oleracea* (Lei et al. 2007; Ze et al. 2011; Kiapour et al. 2015). Although study on wheat (*Triticum aestivum*) plant showed no effect of TiO_2 nanoparticles on RuBisCo activity, total soluble sugar content, photochemical and nonphotochemical quenching values, however the chlorophyll a content and the overall photosystem efficiency faced negative impacts due to the presence of TiO_2. It was also evident that TiO_2 NP builds significant oxidative stress in wheat plant leaves as suggested by Dias et al. (2019).

Reports suggesting silver nanoparticles for agricultural benefits state its role in root development of *Crocus sativus* by blocking the stimulation of ethylene (Rezvani et al. 2012). Silver NP is also known for seedling germination of *Glycine max* by reducing the cytotoxic by-products generation in glycolysis and by enhancing the stress-related protein expression (Mustafa et al. 2015). Certain evidence of silver nanoparticles was reported to favor photosynthesis in *Pelargonium zonale* by the rise in the activity of antioxidant enzymes and the measure of photosynthetic pigments (Ghorbanpour and Hatami 2014). However, Ag-NP at low concentration in *Arabidopsis thaliana* was reported to have raised the amount of oxidative stress generation in the chloroplast, thereby subsequently reducing the production of chlorophyll followed by impairing the photosynthetic elements causing a remarkable decline in the photosynthetic activity and restricting plant growth. It also concluded that diclofop-methyl minimizes the stability of these nanoparticles and its release of harmful oxidative groups remarkably, thus being appropriate for use against silver nanoparticle toxicity (Li et al. 2018).

The variety of responses initiated by zinc oxide NP in plant photosynthesis differs among plant species. For say, one study has reported its positive impacts in photosynthesis in *Helianthus annuus* L. by elevating the assimilation rate of carbon dioxide and chlorophyll content, which increased the stomatal carbon dioxide concentration significantly (Torabian et al. 2016). Similar feedback was observed on *Moringa peregrina* with growth in chlorophyll and carotenoid content due to zinc oxide NP. The chlorophyll content was reported to have increased by threefolds in

Cyamopsis tetragonoloba with the use of zinc oxide NP as foliar (Raliya and Tarafdar 2013). A separate study conducted on *Arabidopsis thaliana* demonstrated the suppressive effects exhibited by zinc oxide NP on the chlorophyll biosynthesis gene like copper response defect 1 (Crd1), chlorophyll synthetase (Chlg), Mg-protoporphyrin IX methyltransferase (Chlm), chlorophyll A oxygenase (Cao), and Mg-chelatase subunit D (Chld). It was also found to inhibit the genes responsible for photosystem I, which includes PS I subunit E2 (Psae2), PS I subunit D2 (Psad2), PS I subunit K (Psak), and PS I subunit N (Psan). Hence, it was concluded that zinc oxide NP acts as a blocking agent of chlorophyll biosynthesis and prevents the photosystem I, thereby reducing the photosynthetic ability of *A. thaliana* (Wang et al. 2016). Drawbacks of zinc oxide NP were reported for *Azolla filiculoides*, where the nanoparticles depleted the contained chlorophyll significantly (Zarate-Cruz et al. 2016).

Copper NP in the different study showed having a toxic effect on photosynthesis in different plant system. One study on *Oryza sativa* showed that copper oxide NP decreased thylakoid number in granum of chloroplast, which reduces its photosynthetic ability to a huge extent. The inhibition of protein expression was observed by copper oxide NP for PSI; however, complete demolish of the PSII was detected with an increase in the applied dose (da Costa and Sharma 2016). Copper oxide NP also minimized the concentration of photosynthetic pigments in *Landoltia punctata* like carotenoids, chlorophyll a, and chlorophyll b (Lalau et al. 2015). However, some reports favored the utilization of copper oxide NP, suggesting their noninterference with chlorophyll content. When exposed to *Elodea nuttallii,* copper oxide NP or copper ions exhibited no toxic effects on photosynthetic pigment even after an exposure period of 24 h (Regier et al. 2015). A similar nondeleterious effect on the photosynthetic pigment was also noted in *Capsicum annuum*; however, the declination in efficiency was observed due to depletion in electron transport capacity of PS II (Rawat et al. 2018).

The level of nanotoxicity induced by gold nanoparticles on plants depends on the coating material used to stabilize the nanostructure. The use of organic compound like citrate or inorganic agents like carbonate to build the coatings for stabilizing agents issued no harmful impacts on the photosynthetic process of *Chlamydomonas reinhardtii* (Behra et al. 2015). But interestingly, when a study conducted on the same species with gold nanoparticle having a coating of monosaccharides like mannose, the photosynthetic yield was decreased to a significant extent (Perreault et al. 2011). *Glycine max* plant showed the dependent chlorophyll loss when gold nanoparticle was applied as well as the efficiency of photosystem I. The reason behind it was predicted as gold NP locks the electrons of photosystem I (Ghosh and Chattopadhyay 2015). However, in recent in vitro study, gold nanoparticles had exhibited positive impact on photosynthesis by enabling the photosystem II for reabsorption of emitted light or photon and increase its efficiency (Torres et al. 2018).

4.2 Nonmetallic Nanoparticle

Nonmetallic nanoparticles like single-walled carbon nanotube (SWCNT), carbon nanodrop, and silicon dioxide nanoparticle are biocompatible and less oxidative, thus having very few toxic effects on plant photosynthesis. Among these nonmetallic nanoparticles, silicon dioxide nanoparticle has a diverse use in agriculture as it boosts germination and seedling growth in different plant species, which include *Lycopersicum esculentum*, *Solanum lycopersicum* L., *Lenus culinaris* Medik., *Cucurbita pepo* L., and *Agropyron elongatum* L. (Haghighi et al. 2012; Azimi et al. 2014; Siddiqui et al. 2014; Sabaghnia and Janmohammadi 2014; Almutairi 2016). Silicon dioxide nanoparticles are also established for the detoxification in plants. For instance, maize plants grown in acidic soil build up internal oxidative stress due to the exposure to the high concentration of aluminum, which degenerates the photosynthetic pigment system of maize. Silicon dioxide nanoparticles used in forms of fertilizer as a foliar not only reduced the generated oxidative stress by aluminum contamination but also seized the aggregation of aluminum in the stromal cells within the photosynthetic apparatus of maize (de Sousa et al. 2019). Studies on *Oryza sativa* and *Triticum aestivum* L. (wheat) have reported similar kind of effects of SiO_2 NPs, where it detoxicated the effect of heavy metal like cadmium accumulation in plants (Ali et al. 2019; Rizwan et al. 2019). These nanoparticles also intensified the photosynthetic rate, stomatal conductance, and synthesis of photosynthetic pigments in *Crataegus* sp. (Ashkavand et al. 2015). Significant elevation of the chlorophyll content in *Ocimum basilicum* was observed, which magnified its photosynthetic efficiency and made competent to survive in high salinity stress (Kalteh et al. 2014). Hence, it can be claimed that SiO_2 NPs have beneficial stress combat application in agriculture as well as enhancement of photosynthetic rate. Other nonmetallic nanoparticles like carbon nanodrops are also reported with a beneficial effect in photosynthesis in mung bean sprout (Wang et al. 2018). SWCNT also showed amplification of photosynthetic efficiency in an in vitro study on chloroplasts (Giraldo et al. 2014). Carbon nanoparticles mainly function by increasing the rate of electron transport within the chloroplast by virtue of their ability to redirect electrons and attachment with biological membranes. Their role in the reduction of photocatalytic or chemo-oxidative stress originated within the chloroplast by providing stability was also evident.

5 Conclusion

The development and emphasis on nanotechnology have produced a variety of nanoparticles with their distinct applications in multitudinous sectors of modern life. But with the increase in its implementation for societal advancement, their release from different anthropogenic sources accumulates in the environment as contaminants. Their typical structural profile makes them highly reactive and stable

in nature, which permits them to persist in the environment for a prolonged period. The exposure of higher concentration of these nanoparticles predominantly has deteriorative effects on biological systems. The current agricultural industry employs these nanoparticles in forms of fertilizers, pesticides, and supplements since their nanoscopic size makes them ideal for absorption within plants. However, the different responses triggered by the applied plants in the presence of these nanoparticles vary among species. The metal-based nanoparticles usually tend to lower the rate of photosynthesis through the generation of oxidative stress within the chloroplast, which depletes the number of photosynthetic pigments contained in the organelle. Nonmetallic nanoparticles like carbon nanodrops, carbon nanotubes, and silicon dioxide promote the photosynthesis mechanism by amplifying the rate of electron transport within the chloroplast or providing a defensive system against the generated oxidative stress. The detailed mechanisms associated with the interactions between the nanoparticles with the photosynthetic system are not well defined and their lies a huge scope of intensive research.

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Chapter 14
Biomedical Applications of Nanoparticles Synthesized from Mushrooms

Mustafa Nadhim Owaid

Contents

1 Introduction

Myconanotechnology is a recent branch in the nanotechnology field which depends on fungal biomass as a reducer and a stabilizer agent to synthesize green metallic nanoparticles (NPs), especially from edible and medicinal mushrooms (Owaid and Ibraheem 2017). Macrofungi/mushrooms grow on the organic substrate in nature (Nivedita et al. 2009). Fungal biomasses include a wide variety of amino acids, proteins, polysaccharides, and phenols existing in mushrooms that are used in the mycosynthesis of both intracellular and extracellular selenium, gold, cadmium,

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silver, and iron NPs (Owaid and Ibraheem 2017) . The biosynthesis of metallic nanoparticles has been carried out from microorganisms and plants such as fungus (Al-Bahrani et al. 2017), yeast (Rahimi et al. 2016), bacterium (Prabhusaran et al. 2016), algae, and plants (Khan et al. 2016; Al-Bahrani et al. 2018; Owaid et al. 2019) . The first study that applied the biosynthesis of nanoparticles from the mushroom started in 2004 when Numata et al. (Numata et al. 2004) produced nanofibers from the polysaccharides/β-1,3-glucan (Schizophyllan) from *Schizophyllum commune* mushroom for the first time, but Vigneshwaran et al. (Vigneshwaran et al. 2007) used the protein of spent mushroom substrates (SMS) of oyster mushroom in 2007 to mycosynthesize the nanoparticle which has a medical importance toward different pathogenic microorganisms. The green chemistry in the field of production of metallic nanoparticle from the mushroom was improved to mycosynthesize eco-friendly AuNPs, AgNPs, and FeNPs, etc., compared to the toxic chemical methods (Owaid and Ibraheem 2017).

2 Mushroom-Mediated Synthesis of Metallic NPs

2.1 Silver Nanoparticles

The synthesized AgNPs from the mushroom have an excellent antibacterial activity against *E. coli* and *P. aeruginosa* compared to *S. aureus* (Nithya and Ragunathan 2009), and thus these AgNPs are effective in controlling the microbes (Balashanmugam et al. 2013). The antibacterial action of mushroom AgNPs was effective against *E. coli*, *K. pneumoniae*, *V. cholera, P. aeruginosa,* and *S. aureus* (Elumalai et al. 2012). However, the biosynthesized AgNPs exhibited antibacterial efficacy against different pathogenic bacteria like *K. pneumoniae* and *S. aureus* (Vigneshwaran et al. 2007) because of their important role in degradation and damaging of cellular macromolecules and DNA of the microbe (Sen et al. 2013a).

2.2 Gold Nanoparticles

The mycosynthesized gold nanoparticles (AuNPs) from the crude extract or glucan of *Pleurotus florida* have been successful (Bhat et al. 2013; Sen et al. 2013a). Mycosynthesis of AuNPs in watery mixture by free-cell filtrate of *P. sapidus* as a reducer and stabilizer agent has an average size of 65 nm (Sarkar et al. 2013). However, polysaccharides of the mushroom *P. florida* were used for biosynthesizing the spherical AuNPs with a rough-cluster surface reached from 5 to 15 nm (Sen et al. 2013a). The reason for the various sizes and shapes of the AuNP is related to the temperature of the reaction (Philip 2009). *Pleurotus ostreatus* has been used to form gold nanoparticles (5–50 nm) from specific proteins (laccase and tyrosinase) (Vetchinkina et al. 2013; El-Batal et al. 2015). These nanoparticles have a spheri-

cal shape with sizes ranging from 22 to 39 nm; they are applied in the field of decolorization of dyes (El-Batal et al. 2015). Fresh and dry aqueous extracts of *Pleurotus cornucopiae* var. *citrinopileatus* (oyster mushroom) are an important selection for reduction of gold ions and in the production of the stable spherical gold nanoparticle ranging from 16 nm to 91 nm and from 23 nm to 100 nm, respectively (Owaid et al. 2017a). Other macrofungi were used to mycosynthesize Ag nanoparticles such as *Terminalia* sp. (the desert truffle) (Owaid et al. 2018) and *Agaricus bisporus* (Atila et al. 2017; Owaid et al. 2017c).

2.3 Selenium Nanoparticles

Also, the selenium nanoparticle (SeNP) has become a new research point since it was found to keep remarkable bioavailability, low toxicity, and excellent anticancer activity. However, SeNPs tend toward aggregation easily and have significant anticancer activity. SeNPs were synthesized from polysaccharide–protein complexes obtained from *P. tuber-regium* sclerotia (Wong 2014). Also, Wu et al. (2012) used polysaccharides–protein complex of mushrooms to synthesize SeNPs and applied them in cellular uptake and anticancer (antiproliferative) efficacy. The anticancer activity is beneficial when using the mushroom *Pleurotus ostreatus* in the synthesis of selenoproteins through the absorption of element of Se from the supplemented media, in vitro (Kaur et al. 2013).

2.4 Iron Nanoparticles

Alternatively, the iron nanoparticles (FeNPs) were significantly biosynthesized inside the hypha of oyster mushroom (*Pleurotus* sp.). The uptake of FeNPs through the fungal cell membrane involves a reduction process from Fe^{+3} (ferric ion) to Fe^{+2} (ferrous ion). Almost all iron uptakes in fungi involve the reduction from the ferric ion to the ferrous ion form using two approaches in two subdivisions, basidiomycota and ascomycota (Mazumdar and Haloi 2011).

2.5 Zinc Sulfide Nanoparticles

Also, oyster mushrooms were used to synthesize zinc sulfide nanoparticles (ZnSNPs) having a size of less than 200 nm (Senapati and Sarkar 2014). The oyster mushroom *Pleurotus ostreatus* was used in the formation of a spherical ZnS NP with sizes ranging from 2 to 5 nm, which has crystalline nature (Senapati and Sarkar 2014). However, Wu et al. (2010) synthesized ZnS-N3 NPs from ubiquitin (protein)

of *P. ostreatus* with diameter average reaching 15 nm, which are applied as desalting probes in protein analysis.

2.6 Cadmium Sulfide Nanoparticles

Fungi were successfully used in mycosynthesis of CdSNPs (Nasrin et al. 2014). The extract of *P. ostreatus* was used to synthesize CdSNPs, which are used in industrial applications. In intracellular, spherical CdS-*Pleurotus* NPs had approximate size of 5 nm (Borovaya et al. 2015), which were used in different industrial applications. *Coriolus versicolor* extract was used with cadmium sulfide to form spherical CdSNPs, which have 100–200 nm (Sanghi and Verma 2009a).

3 Biomedical Applications of Mushroom NPs

3.1 Antibacterial Activity

The biosynthesized silver nanoparticles (AgNPs) from medicinal and edible mushrooms were investigated toward Gram-positive and Gram-negative bacteria, in vitro. The mushroom has antibacterial efficacy (Owaid et al. 2015a); therefore, its fruiting bodies and mycelia are used for the biosynthesis of metallic nanoparticles. The polysaccharide and the protein of oyster mushrooms were used to mycosynthesize AgNPs (Vigneshwaran et al. 2007; Sen et al. 2013b). Polysaccharides and proteins are used as bioreducers and as capping agents, the size them being controllable, and their stability being high (Wong 2014).

Those metallic nanoparticles exhibited antibacterial efficacy toward Gram-positive and Gram-negative bacteria. Intracellularly, the spherical AgNPs were (their diameter ranged from 31 to 100 nm) produced inside mycelia of *Agaricus bisporus, Pleurotus ostreatus, Calocybe indica, Ganoderma lucidum* (Mirunalini et al. 2012), *Pycnoporus sanguineus* (Chan and Don 2013), and *Schizophyllum commune* (Chan and Don 2013; Arun et al. 2014). From another side, extracellularly, both mycelia and fruiting bodies extracts were synthesized and applied as a nanodrug against human pathogenic bacteria, as mentioned in Table 14.1.

Many studies and researchers referred to mycosynthesis of the silver nanoparticle (AgNP) from medicinal and edible mushrooms like *Pleurotus florida* (Bhat et al. 2011), *Pleurotus ostreatus* (Devika et al. 2012), *Pleurotus sajor-caju* (Nithya and Ragunathan 2009; Nithya 2012; Rahi and Barwal 2014), *Pycnoporus sanguineus* (Chan and Don 2013), *Ganoderma lucidum* (Karwa et al. 2011), *Tricholoma crissum* (Ray et al. 2011), *Schizophyllum commune* (Chan and Don 2013; Arun et al. 2014; Sujatha et al. 2016), and *Lentinula edodes* (Sujatha et al. 2015). Moreover, the mycosynthesized AgNPs from the natural extracts of the fungal mycelia (biomass)

Table 14.1 Antibacterial activity of the mushroom nanoparticles and their characteristics

Mushrooms	The used parts	Type of nanoparticles	Shape of NPs	Diameter (nm)	References
Pleurotus sajor-caju	Proteins of SMS	AgNPs	Spherical	30.5	Vigneshwaran et al. (2007)
Pleurotus florida	Polysaccharides	AgNPs	Crystalline	ND	Sen et al. (2013b)
Agaricus bisporus	Mycelia (intracellular)	AgNPs	Spherical	80–100	Mirunalini et al. (2012)
Calocybe indica	Mycelia (intracellular)	AgNPs	Spherical	100	Mirunalini et al. (2012)
Ganoderma lucidum	Mycelia (intracellular)	AgNPs	Spherical	50	Mirunalini et al. (2012)
Pleurotus ostreatus	Mycelia (intracellular)	AgNPs	Spherical	100	Mirunalini et al. (2012)
Pycnoporus sanguineus	Mycelia (intracellular)	AgNPs	Spherical	53	Chan and Don (2013)
Schizophyllum commune	Mycelia (intracellular)	AgNPs	Spherical	54	Chan and Don (2013)
Schizophyllum commune	Mycelia (intracellular)	AgNPs	Spherical	54–99	Arun et al. (2014)
Pleurotus ostreatus	Mycelia (extracellular)	AgNPs	Spherical	8–50	Devika et al. (2012)
Pleurotus florida	Mycelia (extracellular)	AgNPs	Spherical	20	Bhat et al. (2011)
Pleurotus sajor-caju	Mycelia (extracellular)	AgNPs	Spherical	5–50	Nithya and Ragunathan (2009)
Pleurotus sajor-caju	Mycelia (extracellular)	AgNPs	Spherical	35	Nithya (2012)
Pleurotus sajor-caju	Mycelia (extracellular)	AgNPs	Spherical	4–22	Rahi and Barwal (2014)
Pycnoporus sanguineus	Mycelia (extracellular)	AgNPs	Spherical	64–70	Chan and Don (2013)
Lentinula edodes	Mycelia (extracellular)	AgNPs	ND	ND	Sujatha et al. (2015)
Ganoderma lucidum	Mycelia (extracellular)	AgNPs	Various	10–70	Karwa et al. (2011)
Schizophyllum commune	Mycelia (extracellular)	AgNPs	ND	51–93	Arun et al. (2014)
Schizophyllum commune	Mycelia (extracellular)	AgNPs	Spherical	56	Chan and Don (2013)
Schizophyllum commune	Mycelia (extracellular)	AgNPs	Variable	300–500	Sujatha et al. (2016)
Tricholoma crissum	Mycelia (extracellular)	AgNPs	Spherical, hexagonal	5–50	Ray et al. (2011)
Pleurotus ostreatus	Fruiting bodies	AgNPs	ND	50	Elumalai et al. (2012)

(continued)

Table 14.1 (continued)

Mushrooms	The used parts	Type of nanoparticles	Shape of NPs	Diameter (nm)	References
Pleurotus ostreatus	Fruiting bodies	AgNPs	Spherical	100	Mirunalini et al. (2012)
Pleurotus ostreatus	Fruiting bodies	AgNPs	Spherical	40	Al-Bahrani et al. (2017)
Pleurotus florida	Fruiting bodies	AgNPs	ND	ND	Sujatha et al. (2013)
Pleurotus florida	Fruiting bodies	AgNPs	Spherical	5–40	Kaur et al. (2018)
Pleurotus eous	Fruiting bodies	AgNPs	ND	ND	Latha (2010)
Pleurotus platypus	Fruiting bodies	AgNPs	Spherical	560–710	Sujatha et al. (2013)
Pleurotus pulmonarius	Fruiting bodies	AgNPs	ND	ND	Shivashankar et al. (2013)
Pleurotus djamor	Fruiting bodies	AgNPs	ND	ND	Shivashankar et al. (2013)
Pleurotus giganteus	Fruiting bodies	AgNPs	Spherical	5–25	Debnath et al. (2019)
Phellinus igniarius	Fruiting bodies	AgNPs	Spherical	<100	Paul et al. (2015b)
Hypsizygus ulmarius	Fruiting bodies	AgNPs	ND	ND	Shivashankar et al. (2013)
Calocybe indica	Fruiting bodies	AgNPs	ND	ND	Sujatha et al. (2013)
Ganoderma lucidum	Fruiting bodies	AgNPs	Spherical	50–100	Mirunalini et al. (2012); Paul et al. (2015b)
Ganoderma lucidum	Fruiting bodies	AgNPs	Face centric cubic	75	Paul et al. (2015a)
Ganoderma applanatum	Fruiting bodies	AgNPs	Spherical	133–0.36	Mohanta et al. (2016)
Inonotus obliquus	Fruiting bodies	AgNPs	Spherical	14–35	Nagajyothi et al. (2013)
Microporus xanthopus	Fruiting bodies	AgNPs	Spherical	40	Balashanmugam et al. (2013)
Tricholoma matsutake	Fruiting bodies	AgNPs	Spherical	10–20	Anthony et al. (2014)
Agaricus bisporus	Fruiting bodies	AgNPs	Spherical	10–20	Narasimha et al. (2011)
Agaricus bisporus	Fruiting bodies	AgNPs	ND	ND	Dhanasekaran and Latha (2013); Sujatha et al. (2013)
Agaricus bisporus	Fruiting bodies	AgNPs	Spherical	30	Sudhakar et al. (2014)

(continued)

Table 14.1 (continued)

Mushrooms	The used parts	Type of nanoparticles	Shape of NPs	Diameter (nm)	References
Agaricus bisporus	Fruiting bodies	AgNPs	Dispersed	20–44	Ul-Haq et al. (2015)
Agaricus bisporus	Fruiting bodies	AuNPs	ND	33.5–0.8	Eskandari-Nojehdehi et al. (2016)

ND non-detected

have a spherical shape and they show high efficacy against the pathogenic bacteria in vitro.

Also, the natural extracts of mushrooms' fruiting bodies were used as green reducers to produce silver nanoparticles (AgNP) and gold nanoparticles (AuNP), as given in Table 14.1. AuNPs were produced from *Agaricus bisporus* and applied as antibacterial agents (Eskandari-Nojehdehi et al. 2016). Otherwise, AgNPs were produced from various mushrooms like *Pleurotus ostreatus* (Elumalai et al. 2012; Mirunalini et al. 2012; Al-Bahrani et al. 2017), *Pleurotus florida* (Sujatha et al. 2013; Kaur et al. 2018), *Pleurotus eous* (Latha 2010), *Pleurotus platypus* (Sujatha et al. 2013), *Pleurotus pulmonarius, Pleurotus djamor, Hypsizygus ulmarius* (Shivashankar et al. 2013), *Pleurotus giganteus* (Debnath et al. 2019), *Phellinus igniarius* (Paul et al. 2015b), *Calocybe indica* (Sujatha et al. 2013), *Ganoderma lucidum* (Mirunalini et al. 2012; Paul et al. 2015b), *Inonotus obliquus* (Nagajyothi et al. 2013), *Microporus xanthopus* (Balashanmugam et al. 2013), *Tricholoma matsutake* (Anthony et al. 2014), and *Agaricus bisporus* (Narasimha et al. 2011; Dhanasekaran and Latha 2013; Sujatha et al. 2013; Sudhakar et al. 2014).

The antibacterial activity of the mycosynthesized oyster mushroom AgNPs was investigated by Mirunalini et al. (Mirunalini et al. 2012) against *S. aureus* and they observed a good inhibition zone. The silver nanoparticles (AgNPs) are known to be good anti-inflammatory and antibacterial agents, and are thus applied to enhance wound healing (Fu et al. 2006). The antibacterial mechanisms of Ag^+ are not exactly known until now and may be derived through the electrostatic attraction between the negative charge of bacterial cell membranes and the positive charge of silver nanoparticles (Dibrov et al. 2002). Thus, the mycosynthesized AgNPs were selected as a suitable nanodrug against different pathogenic bacteria.

3.2 Antifungal Activity

The silver nanoparticles (AgNPs) biosynthesized from edible and medicinal mushrooms were tested against fungi (molds and yeasts) in vitro. The mushroom has antifungal efficacy (Owaid et al. 2017b) and thus their mycelia and fruiting bodies are used to biosynthesize metallic nanoparticles.

These nanoparticles were synthesized intracellularly and extracellularly of mycelia and from fruiting bodies. Intracellularly, the spherical AgNPs were (their

Table 14.2 Antifungal activity of mushroom nanoparticles and their characteristics

Mushrooms	The used parts	Type of nanoparticles	Shape of NPs	Diameter (nm)	References
Schizophyllum commune	Mycelia (intracellular)	AgNPs	Spherical	54–99	Arun et al. (2014)
Schizophyllum commune	Mycelia (extracellular)	AgNPs	ND	51–93	Arun et al. (2014)
Pleurotus ostreatus	Mycelia (extracellular)	AgNPs	Spherical	4–15	Yehia and Al-Sheikh (2014)
Tricholoma crissum	Mycelia (extracellular)	AgNPs	Spherical and hexagonal	5–50	Ray et al. (2011)
Pleurotus cornucopiae var. *citrinopileatus*	Fruiting bodies	AgNPs	Spherical	20–30	Owaid et al. (2015b)
Pleurotus cornucopiae var. *citrinopileatus*	Fruiting bodies	AgNPs	Spherical	10–50	Owaid (2013)
P. sajor-caju	Fruiting bodies	AgNPs	Spherical	17	Musa et al. (2017)

ND non-detected

diameter ranged from 54 to 99 nm) produced inside mycelia of *Schizophyllum commune* and they exhibited antidermatophytic fungal activity (Arun et al. 2014). From another side, extracellularly, both mycelia and fruiting bodies' extracts were synthesized and exhibited antiplant pathogenic fungal activity, antidermatophytic fungal activity, and anticandidal activity as shown in Table 14.2.

Many researchers reported mycosynthesis of AgNPs with antifungal efficacy from edible and medicinal mushrooms such as *Schizophyllum commune* (Arun et al. 2014), *Pleurotus ostreatus* (Yehia and Al-Sheikh 2014), *Tricholoma crissum* (Ray et al. 2011), *Pleurotus cornucopiae* var. *citrinopileatus* (Owaid 2013; Owaid et al. 2015b), and *P. sajor-caju* (Musa et al. 2017). However, the biosynthesized AgNPs from the extracts of fungal mycelial (biomass) were spherical in shape and exhibited high effect toward *Candida* spp. in vitro. The results of antifungal activity showed that AgNPs have moderate inhibitory activity against *C. pseudotropicalis, C. glabrata*, *C. albicans,* and *C. krusei* infections, in vitro (Owaid et al. 2015b). Also, the antifungal efficacy of the AgNP toward the yeast *Candida albicans* was reported by Yehia and Al-Sheikh (Yehia and Al-Sheikh 2014).

Some researchers have referred that the positive charges of silver element are crucial for their antifungal activity through cell permeability and progressive release of membrane constituents (Sastry et al. 1997), free radical generation (Sanghi and Verma 2009b), and the electrostatic attraction between the negative charge of the fungal cell membrane and the positive charge of the AgNP (Janga et al. 2011; Meng et al. 2011).

Table 14.3 Antioxidant activity of the mushroom nanoparticles and their characteristics

Mushrooms	The used parts	Type of nanoparticles	Shape of NPs	Diameter (nm)	References
Ganoderma lucidum	Fruiting bodies	AgNPs	Spherical	<100	Paul et al. (2015b)
Phellinus igniarius	Fruiting bodies	AgNPs	Spherical	<100	Paul et al. (2015b)
Inonotus obliquus	Fruiting bodies	AgNPs	Spherical	14–35	Nagajyothi et al. (2013)
Agaricus bisporus	Fruiting bodies	Chitosan	ND	ND	Dhamodharan and Mirunalini (2012, 2013)

ND non-detected

3.3 Antioxidant Activity

In general, the mushroom has antioxidant efficacy that leads to raise its anticancer and antitumor activities (Chang and Miles 2004; Patra et al. 2013). This phenomenon is useful to mycosynthesize the metallic NP, which has a positive antioxidant property that reflects on the potential anticancer characteristics (Owaid and Ibraheem 2017). The mycosynthesized Ag nanoparticle from various mushrooms leads to its application as a nanodrug because of its high anticancer activity and low toxicity to the normal cell in vivo compared with the chemo-synthesized Ag nanoparticle (Egorova et al. 2016). Fruiting bodies of *Agaricus bisporus* (Dhamodharan and Mirunalini 2012, 2013), *Ganoderma lucidum*, *Phellinus igniarius* (Paul et al. 2015b) and *Inonotus obliquus* (Nagajyothi et al. 2013) were used to synthsize nanoparticles which had antioxidant activity, as in Table 14.3.

3.4 Anticancer Activity

The mycosynthesized metallic nanoparticles, such as AuNPs (Bhat et al. 2013), AgNPs (Ismail et al. 2015), and SeNPs (Wu et al. 2013b), are considered a potential nanodrug against many cancer cell lines that have been investigated and applied. Polysaccharides–protein complex of some mushrooms like *Pleurotus tuber-regium and Polyporus rhinocerus* was used to biosynthesize SeNPs (Wu et al. 2013a; Wong 2014). Mycelia (intracellular) and mycelia (extracellular) of the mushroom *Schizophyllum commune* were used to mycosynthesize AgNPs (Arun et al. 2014), while only mycelia (extracellular) of *Pleurotus ostreatus* (Yehia and Al-Sheikh 2014) and *Ganoderma neo-japonicum* (Gurunathan et al. 2015) were used in the previous work as in Table 14.4.

The extracts of fruiting bodies of mushrooms also were used as a green reducer to produce AgNPs and gold nanoparticles (AuNPs) as in Table 14.4. AuNPs were produced from *Pleurotus florida* (Bhat et al. 2013) and *Hericium erinaceus* (Raman et al. 2015) and applied as anticancer agents. Otherwise, AgNPs were produced

Table 14.4 Anticancer activity of the mushroom-nanoparticles and their characteristics

Mushrooms	The used parts	Type of nanoparticles	Shape of NPs	Diameter (nm)	References
Mushroom	Polysaccharides–protein complex (PPC)	SeNPs	Spherical	<50	Wu et al. (2012)
Pleurotus tuber-regium	PPC	SeNPs	ND	ND	Wong (2014)
Polyporus rhinoceros	PPC	SeNPs	Spherical	ND	Wu et al. (2013a)
Schizophyllum commune	Mycelia (intracellular)	AgNPs	Spherical	54–99	Arun et al. (2014)
Schizophyllum commune	Mycelia (extracellular)	AgNPs	ND	51–93	Arun et al. (2014)
Pleurotus ostreatus	Mycelia (extracellular)	AgNPs	Spherical	4–15	Yehia and Al-Sheikh (2014)
Ganoderma neo-japonicum	Mycelia (extracellular)	AgNPs	Crystalline	<6	Gurunathan et al. (2015)
Inonotus obliquus	Fruiting bodies	AgNPs	Spherical	14–35	Nagajyothi et al. (2013)
Pleurotus djamor var. *roseus*	Fruiting bodies	AgNPs	Spherical	5–50	Ramana et al. (2015)
Pleurotus ostreatus	Fruiting bodies	AgNPs	Spherical	17.5	Ismail et al. (2015)
Phellinus igniarius	Fruiting bodies	AgNPs	Spherical	<100	Paul et al. (2015b)
Ganoderma lucidum	Fruiting bodies	AgNPs	Spherical	<100	Paul et al. (2015b)
Pleurotus florida	Fruiting bodies	AuNPs	Irregular, spherical, triangular	10–50	Bhat et al. (2013)
Hericium erinaceus	Fruiting bodies	AuNPs	Spherical	20–40	Raman et al. (2015)

ND non-detected

from various mushrooms like *Pleurotus ostreatus* (Ismail et al. 2015), *Pleurotus djamor* var. *roseus* (Ramana et al. 2015), *Inonotus obliquus* (Nagajyothi et al. 2013), *Phellinus igniarius*, and *Ganoderma lucidum* (Paul et al. 2015b).

The gold nanoparticle (AuNP) showed remarkable dose-dependent antiproliferative effect toward different cancer cell lines due to its irregular shape and its functionalization with organic moieties. The potential applications of the mycosynthesized gold nanoparticle (AuNP) from *P. florida*, magnetite nanoparticle, and other inorganic nanoparticles in hyperthermia of cancer cells were investigated by Bhat et al. (Bhat et al. 2013).

However, The AgNPs of *Pleurotus sanguineus* with a size of 20 nm have more cytotoxic than ions of silver. Also, the smallest particle size of AgNP has been recorded to have a greater ability for apoptosis induction in Mc3T3-E1 cell line than

the larger AgNP. Thus, the design of nanoparticles sizes needs to be done carefully for use in biomedical and pharmaceutical applications (Chan and Don 2013). The anticancer characteristics of silver nanoparticles have been tested against MCF7 cells (breast carcinoma cells). They caused a remarkable decrease in the cell viability of MCF7 cell lines and inhibited the growth of cells up to 78% depending on the AgNPs dose (Yehia and Al-Sheikh 2014).

4 Conclusion and Future Prospects

This chapter aims to distinguish synthesizing metallic nanoparticles from edible and medicinal mushrooms in terms of so-called "myconanotechnology." Mycomaterials have been used as a mycoreducer to produce green metallic nanoparticles. Mycomaterials include crude extracts like extracts of fruiting bodies, fungal mycelia, and free cell filtrate, or purified matters like polysaccharides, enzymes/proteins, and polysaccharide–protein complexes. Green chemistry methods have attempted to mycosynthesize AgNPs, AuNPs, FeNPs, SeNPs, CdSNPs, ZnSNPs, and PaNPs using mycological materials by various approaches. The green mushroom nanoparticles (mushroom NPs) have been investigated as antibacterial, antifungal, anticandidal, antioxidant, anticancer, and antitumor agents. Generally, *Pleurotus* AgNPs have a higher synthesis and wider therapeutic applications among mushrooms. The medical role of all synthesized nanoparticles is due to their unique characteristics such as nanosize, crystalline nature, and eco-friendly agents.

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Chapter 15
Green Synthesis of Nanoparticles by Mangrove Plants and Its Biomedical Application

Ruchi Rathod and Bhawana Pathak

Contents

1 Introduction

Nanotechnology is a rapidly developing area of research with chemical, biological, physical, and engineering sciences interspersed (Gouda et al. 2015). Nanotechnology is mainly focused on creating nanostructures such as metal nanoparticles, graphene, and their composites, carbon nanotubes (CNTs) and quantum dots (QDs) (Singh et al. 2018). It is a science centered on atomic, molecular, and supermolelcular chemistry that involves the synthesis, design, and manipulation of particle size that ranges from 10 to 100 nm (Moodley et al. 2018; Roy 2017). Nano scale sizes have an advantage of significant enormous surface-to-volume ratio and expanded surface area, and enhanced physical and chemical properties of NPs in solution (Abdi et al. 2018). Due to its unique properties, nanoparticles show applications in healthcare, environment, chemical industries, optics, etc. Nanobiotechnology deals with the biological systems that investigate the use of nanoparticles. It also provides different techniques to synthesize environment-friendly, nontoxic, and clean technology

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for the synthesis of metal nanoparticles. Nanoparticles possess specific properties like size, distribution, and morphology in comparison to larger particles (Roy 2017).

Nano is utilized to explain one billionth of a meter. Nano is a Greek phrase that means extremely small. The term nanotechnology was instituted by Professor Norio Taniguchi in 1974 to explain the production of materials at the nanometric level. Nanotechnology is a rapidly developing field with its application to formulation of nanoscale-level materials in the area of innovation and science.

It is a multidisciplinary scientific challenge, and entails creation and utilization of substances capabilities at nanoscale. Nano phasic and nanostructured materials are attracting more attention due to their specific process which makes their applications in biological and pharmaceuticals. More than this, it also covers the pollution sensing through various techniques, and helps in environmental risk assessment and monitoring. It is also applied in cosmetic, electronic, and energy-related applications.

Nanoparticle can be said as bridge between bulk materials and molecular structures. Bulk materials have constant physical properties because they have grain structures with random grains individually oriented in space and connecting each other across grain boundaries but nanomaterials are made up of a single grain with all the atoms oriented in crystalline lattice.

Nanoparticles indicate various properties such as surface plasma resonance, quantum confinement, and melting temperature decrease, which might be immediately identified with the crystalline lattice of the nanomaterials (Sharma et al. 2009). The nanoparticles are fundamentally characterized into two classes: inorganic nanoparticles and organic nanoparticles. Inorganic nanoparticles are noble nanoparticles, for instance, silver and gold nanoparticles; semiconductor nanoparticles, for example, zinc oxide and titanium oxide; and organic nanoparticles incorporate carbon nanoparticles, for instance, fullerenes. Silver nanoparticles are utilized in wide scope of utilizations in beautifying agents, pharmaceuticals, medical devices, clothing, and water refinement.

In nature, numerous metals are present, but only a few of them are synthesized in nanostructure like gold, zinc, palladium, platinum, and silver (Pirtarighat et al. 2019). Among those mentioned above, silver nanoparticles attract attention due to their unique properties such as morphology, particular geometry, and stability (Shaik et al. 2018). Application of AgNPs includes agriculture, air filtration, water purification, and pharmaceuticals (Pirtarighat et al. 2019). AgNPs are also used in coating materials, molecular switches, data packing, and sensing devices. Furthermore, AgNPs possess extremely good antimicrobial activities against some microorganism. Silver nanoparticles have superior antimicrobial activity to other metals such as copper, mercury, lead, and chromium (Premasudha et al. 2015).

Numerous methods for the synthesis of nanoparticles are developed to improve the properties and some modified methods are developed to achieve specific nanoparticles. The synthesis of nanoparticles is classified into two methods: bottom-up and top-down (Ealias and Saravanakumar 2017). The construction method or the bottom-up method includes the accumulation of materials from the base: atom by atom, molecule by molecule, or group by group. This procedure is mostly

used to create nanoscale materials with the ability to produce a uniform shape, size, and distribution. Spinning, Sol gel, pyrolysis, biosynthesis, and chemical vapor deposition (CVD) are the most commonly used bottom-up methods for nanoparticle synthesis. The destructive or top-down method refers to a reduction in bulk materials and makes it smaller to particles on a nanometric scale. Laser ablation, nanolithography, sputtering, thermal decomposition, and mechanical milling are among the broadly used nanoparticle synthesis techniques (Ealias and Saravanakumar 2017).

The physical methodologies are evaporation, condensation, and laser ablation. Nonattendance of solvent contamination in prepared nanoparticles is an advantage of physical method in contrast with the chemical method. The physical method of a tube furnace at atmospheric pressure has a few drawbacks like more power consumption, a huge space for tube furnace, and a large amount of energy. Some studies have shown a synthesis of silver nanoparticles by way of a small ceramic heater with a local heating region. This physical method is useful to synthesize small NPs in high concentration. The laser ablation of bulk metal materials is also used for silver NPs. The productivity of synthesized nanoparticles relies upon a few parameters, for example, wavelength of laser, the laser fluence, length, and duration, etc. From these techniques, unadulterated and uncontaminated metal colloids can be synthesized as nonappearance of chemical compounds in a solution (Iravani et al. 2014).

The chemical method includes the synthetic decrease by organic and inorganic reducing dealers for synthesis of different nanoparticles. For the reduction of silver ions to silver nanoparticles, different reducing agents are used such as sodium citrate, Tollens reagent, N, N-dimethylformamide (DMF), and sodium borohydride ($NaBH_4$) solutions. To keep away from the agglomeration of synthesized metal nanoparticles, surfactants are used that protect the particles from the loss of their surface properties and sedimentation. It has been reported that polymeric mixes are protective agents for stabilizing nanoparticles (Iravani et al. 2014). Physical and chemical strategies are costly and non-eco-friendly. Therefore, these chemical and physical methods are facing challenges and inspiring the researchers to find an alternative way to synthesize NPs.

In this context, biological methods are ecological and cost-effective for the synthesis of nanoparticles. As a reducing agent, plants and microorganisms are used. The biogenic synthesized nanoparticles are highly stable and have good properties. Several studies reported the fungi, bacteria, and algae utilized for synthesis of biogenic nanoparticles (Iravani et al. 2014).

Green plant-mediated nanoparticles have received more attention in evaluation with chemical and physical methods. This method is environmentally friendly, cost-effective, and safe for biomedical applications (Iravani et al. 2014). Also, they offer a large-scale production of nanoparticles because of low-cost advantages. Apart from this, it reduces the labor in the maintenance of cell culture. Extracts of plants go about as a capping and reducing agent for green nanoparticles synthesis. The presence of secondary metabolites (alkaloids, flavonoids, phenols, tannins, terpens, etc.) in plant extracts is responsible for the core mechanism of reduction. Figure 15.1 shows the different approaches of nanoparticle synthesis.

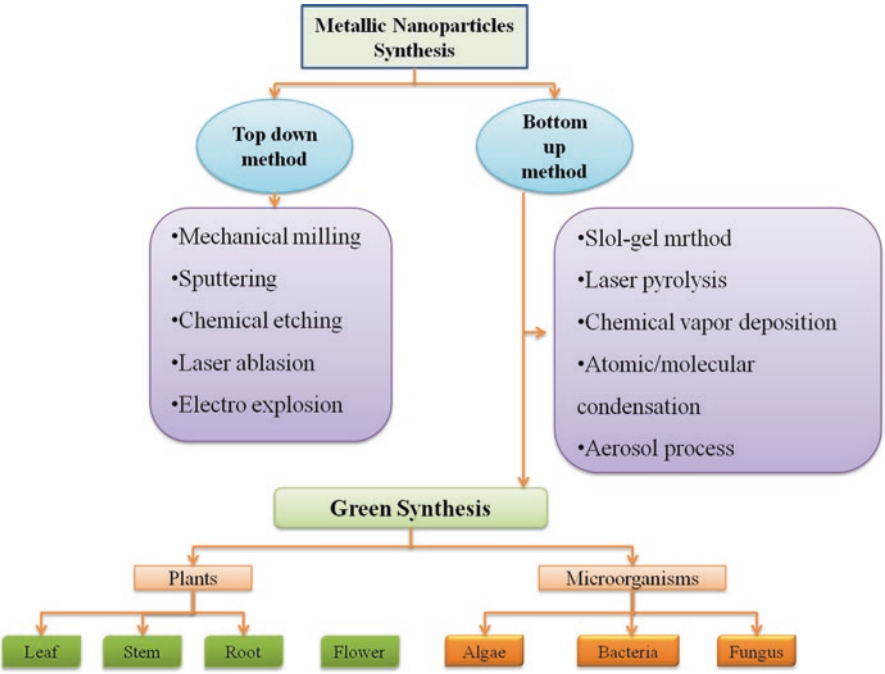

Fig. 15.1 Different approaches of nanoparticles synthesis

It is well known that mangroves and mangrove associates are abundant in secondary metabolites due to their unique environmental conditions (Lanka 2017). There are restricted investigations that reveal the synthesis of green nanoparticles utilizing mangroves (Abdi et al. 2018).

Mangroves trees are halophytes, a plant that thrives in salty conditions. They develop in conditions where no other vascular plants can develop productively; this makes a significant contribution that benefits the environment (Mangrove.org). The term mangrove was referred by Tomlinson (1986) (Naik and Dhabe 2018). The total mangrove forest cover in India is about 4662.56 km^2 while globally they cover around 1,46,500.00 km^2. This represents 3% of the global mangrove area and 0.14% of the total geographical area of the country (Devi and Pathak 2016).

Mangroves are most gainful and naturally significant biological systems of the world since it gives significant and uncommon ecosystem good services and other supportive features to human culture, coastal, and marine systems. Mangroves have high economic and ecological importance because they allow to stabilize seashores and lessen the overwhelming effects of natural disasters like hurricanes and tsunamis, reproducing nursing grounds to marine species as well as food, gasoline, medicinal drug, and constructing fabric for local communities. The fast degradation and disappearance of mangroves ought to have bad outcomes on the marine system and impact the atmospheric composition and weather (Giri et al. 2011).

Mangroves forests survive in extreme situations like excessive temperatures, high saltiness, low oxygen, and muddy soil (Devi and Pathak 2016). Mangroves have different habitat strategies to survive in harsh environment like adjustments to low oxygen, restricting salt admission, limiting water loss, nutrient uptake and expanding survival of offspring (Patra and Mohanta 2014). They have developed different chemophysiological processes to reproduce and survive in unique environments and habitats. Such a metabolic process of mangrove plants stimulates the synthesis of various secondary metabolites. These secondary metabolites utilized as bioactive compounds also serve as anticancerous, antimicrobial, antifungal, and antiviral medicines, etc. (Table 15.1). The capacities of mangrove plants to produce

Table 15.1 Medicinal properties of different mangrove species

Sr. No.	Mangrove species	Plant part used	Medicinal properties	References
1.	*Rhizophora apiculata*	Stem, bark	Antioxidants	
2.	*Rhizophora mucronata*	Leaves, bark	Elephantiasis, hematoma, hepatitis, ulcers, and a febrifuge, bark-powerful, astringent useful in diabetics, hemorrhage	Revathi (2013)
3.	*Acanthus illicifolius*		Paralysis, asthma, rheumatic pains, analgesics, anti- inflammatory and leishmanicidal activities, anticancer and anti-viral agents, used for reducing the poison snakebite, skin diseases, kidney stone, smallpox and ulcer	Bandaranayake (2002) Revathi (2013)
4.	*Aegicerasc orniculatum*		Asthma, diabetes, rheumatism	Revathi (2013)
5.	*Avicennia marina*	Leaves	Rheumatism, small pox, ulcers	Revathi (2013)
6.	*Avicennia officinalis*	Leaves	Small pox, joint pain, urinary disorders, bronchial asthma, stomach disorders, as an aphrodisiac, diuretic, hepatitis, leprosy	Revathi (2013)
7.	*Bruguiera cylindrica*		Hepatitis	Revathi (2013)
8.	*Bruguiera gymnorhiza*	Fruit root & leaves,	Eye diseases, diarrhea, root & leaves- to treat burns	Bandaranayke (1998) Bamroongrugsa (1999)
9.	*Ceriops decandra*		Hepatitis, ulcer	Revathi (2013)
10.	*Excoecaria agallocha*		Uterotonic, purgative, treatment of epilepsy, conjunctivitis, dermatitis, hematuria, leprosy, toothache	Revathi (2013)
11.	*Lumnitzera racemosa*		Antifertility, treatment of asthma, diabetes, snakebite	Revathi (2013)
12.	*Sonneratia apetala*	Leaves	Hepatitis	Revathi (2013)

such unique molecules encourage the researchers to discover significant medications for some potent therapeutic products (Patra and Mohanta 2014). In the adverse environmental stress conditions, mangroves produce different constituents like alkaloids, phenols, tannins, flavonoids, and saponins that possess different antimicrobial activities. Much research is undertaken for the discovery of biological activities of the mangrove plant. Disk diffusion assays, agar well diffusion assays, dilution assays, and bioautographic assays have been used to investigate the antimicrobial activities of mangroves. The most widely recognized methods of activity for antimicrobial activities are interfering with nucleic acid, interfering with cell wall and cell membrane, and enzyme interactions, etc. (Patra and Mohanta 2014). The major compounds that are involved in antimicrobial activities are quinons, flavones, flavonoids, flavonols, tannins, terpens, polypeptides, and alkaloids. Mangroves are a novel source of development of novel drugs (Patra and Mohanta 2014).

The mangroves offer forestry (charcoal, firewood, honey, wood, and many others.) and fishery products (crab, mollusk, fish, prawn, and so forth.). The twigs of mangroves are used for preparing firewood and charcoal because of its excessive calorific values. Mangrove forests are extraordinarily crucial coastal assets, which can be crucial to our socio-socio-economic development. The mangroves are resources of fairly valued business merchandise and fishery assets and also as websites for developing eco-tourism. The forests of mangroves have been appeared to continue extra than 70 direct human activities, which includes gas-wood collection to fisheries (Kathiresan and Bingham 2001). Mangroves offer accurate area to honey bees and assist to facilitate apiculture activities. In the Sundarban area, it provides employment to over 2000 people, who extract a large amount of honey annually (Krishnamurthy 1990). Avicennia and other mangroves are also used as a meals supplement for camel, sheep, buffaloes, and other cattle in India, the Persian Gulf area, Pakistan, and Indonesia (Mukhtar and Hannan 2012).

2 Biomedical Application of Green Synthesized Nanoparticles

2.1 Drug Delivery

For drug therapy, nanoparticles have been used. To the targeted tissue, the appropriate dose of drugs could be reached, but to ensure the highest efficiency with patient's safety, they are engineered to deliver in an arranged time span. Gold nanoparticles are used to prepare scaffolds and vehicles for medication conveyance because of its nontoxicity, nonimmunogenicity, and functionalization properties. The different states of nanoparticles react to different infrared wavelengths, for instance, nanocapsules, and nanobones are dissolved at light wavelengths compared to 1100 and 800 nm, separately. Au nanoparticles are also used for cancer therapy (Razavi et al. 2015). ZnO nanoparticles are also used for the drug delivery system due to their basic properties. ZnO nanoparticles can enter through little vessels (smaller capillaries) due to their

smaller size and consumed by using the cells for an effective amassing of medications to the focused areas. Moreover, for preparation of nanoparticles, biodegradable materials were utilized, which allows the delayed release of medications at the engaged site over a period of days or even weeks (Kalpana and Devi Rajeswari 2018).

2.2 Bioimaging

The plasmonic properties of silver nanoparticles can be distinguished by different optical microscopy procedures and it is valuable over other utilized fluorescent dyes that break down during imaging. AgNPs are extensively utilized as biological probes to reveal dynamic events due to their photostable properties. Moreover, researchers also reported the ongoing investigation of silver nanoparticles to observe early embryonic development. The small metallic nanoparticle of AgNPs also encourages its uses as therapeutic tools. AgNPs are employed in bioimaging in two ways. First of all is the incubation of silver nanoparticles with cells in order to check the uptake and physical interaction and the second is on the surface of AgNPs, the functionalization of biomolecule since it increases the specificity of the cell membrane (Khatoon et al. 2017). There are numerous reports that demonstrate the usage of ZnO nanomaterials for cell imaging as ZnO nanomaterials contain basic excitonic blue and near UV emission, which has green luminescence associated with O_2 vacancies. Green fluorescent ZnO NPs conjugated with transferrin were used to obtain images of cancer cells, as they have the capacity to infiltrate into the cell core (Kalpana and Devi Rajeswari 2018).

2.3 Biosensors and Labeling

Biosensors are broadly utilized in ecological observing, sustenance industry, social insurance, and in chemical or biological examination. Photometric, electrochemical, calorimetric, and piezoelectric are examples of biosensors which are categories on the detection principles (Kalpana and Devi Rajeswari 2018). Metal nanoparticles are used as biosensors due to their high surface region, which could be used for immobilizing biomolecules like catalysts, antibodies, and so forth, and they give a wide way to deal with the improvement of electronic and optical biosensors (Razavi et al. 2015; Kalpana and Devi Rajeswari 2018). Metal nanoparticles like gold and silver show plasmon absorbance bands in the visible spectra region and this is constrained by particles size. Binding to special molecules changes their optical behaviors which allow the ion quantification and detection of analytes. Au nanoparticles change their absorption properties when agglomeration occurs. Moreover, researchers also used metal particles for bioassay labeling and tissue staining as a way to deal with the observing natural (biological) process (Razavi et al. 2015). The plasmonic properties of silver nanoparticles primarily rely upon the shape, size, and dielectric

medium that encompass it, and this makes it appropriate for biosensing. For sensing different interactions, the distinctively formed AgNPs are fused in biosensors. The triangular AgNP manufactured by Haes and van by nanosphere lithography are covered on glass substrate and those surface-covered nano biosensors are utilized to find out interactions between biomolecules, for example, biotin-streptavidin and two biomolecules which are responsible for Alzheimer's disease. The rhombohedral or cubical AgNPs were used for sensing protein interaction. Furthermore, AgNPs-based biosensors are also used in cancer detection and additionally studies also showed the utilization of silica-coated nanosilver as biosensors for the detection of bovine serum albumin (Khatoon et al. 2017).

Metal nanoparticles have electron absorbing properties which are used to produce contrast. Gold nanoparticles have highly absorbing electron capacity which makes it suitable for use as a contrasting agent in transmission electron microscopy. In addition, they are utilized for biotagging or labeling, as they have the same size of proteins. Au nanoparticles provide a very high spatial resolution and thus utilized for some of labeling applications, that is, with antibodies (immunostaninng) (Razavi et al. 2015).

2.4 Medicine and Dentistry

Nanoparticles also demonstrate the antimicrobial, antibacterial, antiviral, and antifungal, properties, etc. due to their enormous surface region. Metallic nanoparticles have the capacity to adequately repress the development of numerous microorganisms, therefore expanding their application in medication and dentistry (Razavi et al. 2015). Numerous examinations exhibited the antifungal action of silver nanoparticles against *Candida* species, for example *C. krusei*, *C. glabrata*, *C. albicans*, and *C. parapsilosis.* In conclusion, AgNPs synthesized using *Ocimum sanctum L.* (Tulsi) indicated antifungal movement against an entrepreneurial human parasitic pathogen. The cytoprotective properties of silver have been utilized for avoidance of HIV cooperation to the host cells. Additionally, it is used to avoid contamination after a medical procedure and as against HIV-1 agent (Khatoon et al. 2017). In dental materials, nanoparticles can be utilized as antibacterial agents. Titanium is broadly utilized in the domain of dentistry due to its ductility and high fracture resistance; however, it does not support cell bond and development because of its absence in bioactivity. In the past, apatite covering on titanium was utilized because of their capacity to bond with bone and bioactivity; yet, nonuniformity and thickness of apatite are considered as constraints. In addition, for nutrient transport, permeable structures are required. Ceramic nanoparticles are utilized to configure an artificial hybrid material that could be placed on the surface of the tooth to improve scratching. Nanosized features in bone implants are also an emerging approach, as they reduce the chances of rejection on the surface of the prosthesis because they stimulate the production of osteoblasts (Razavi et al. 2015).

Furthermore, silver nanoparticles are also utilized in medical devices such as catheters, bone cement, and wound healing. Silver nanoparticles are broadly used in topical treatments, such as in creams used as an antiseptic to cure infection and wounds. In addition, they are also used in implants and medical devices. Catheters coated with nanoparticles are biocompatible, as they have an inclination to discharge explicitly and support arrival of silver at the implantation site (Khatoon et al. 2017). Metal nanoparticles are likewise utilized for water decontamination and expulsion of pesticides from drinking water.

3 Synthesis of Nanoparticles by Mangroves and Their Application

The nanoparticles are utilized in different areas of technology, science, and engineering, from optics to biomedicine, paints, biosensors, horticulture, materials, and search engines. They are in each field of innovation and progress. Studies state that a broad spectrum of nanoparticles has been synthesized by several biogenic strategies that use natural substrates and reducing agents. Nanoparticles that have been combined thus far and efficaciously consolidated in distinctive biomedical, agrarian, and present-day programs consist of gold (Au), iron (Fe), copper (Cu), lead (Pd), silver (Ag), lead (II) sulfide (PbS), copper (II) oxide (CuO), ruthenium (Ru), cadmium sulfide (CdS), zinc oxide (ZnO), and titanium dioxide (TiO2). These applications are constrained to utilize a greater part of the nanoparticles obtained from earthbound life forms, abandoning the nanoparticles obtained from living beings from the mangrove environment. Because of the unforgiving natural conditions, the creatures from these areas have created different pressure-tolerant mixers and biotics of excessive ethnobotanical significance. As indicated by one estimate, mangrove soil sequesters roughly 22.8 million large metric amounts of carbon every year. This important coastal area is explored and the presence of bioactive compounds in these mangrove plants for synthesizing nanoparticles results in their application in the biomedical field (Gouda et al. 2019).

Mangroves were utilized in medications and extracts of mangrove species have demonstrated inhibitory action against plant pathogens, creatures, and humans. A few types of mangrove produce bioactive compounds that can control microbial development. Additionally, fundamental investigations have exhibited that the extracts of mangrove plants have antibacterial action against some strains of pathogenic bacteria; Escherichia coli, Staphylococcus sp., Pseudomonas sp., and antimicrobial-resistant bacterial strains; Proteus sp., and Staphylococcus sp. Extracts of mangroves can likewise be the potential source of mosquito larvicides, anticancer, antiviral, antifungal agents and against diabetic compounds. Secondary compounds such as steroids, alkaloids, terpenoids, and phenolics were described from mangroves and their associates possess pharmacological, toxicological, and natural significance (Saranraj and Sujitha 2015).

Mangroves are the potential source of nanoparticle synthesis, as they survive in extremely different conditions from terrestrial plants and have proven antimicrobial activities (Gouda et al. 2015). The first mangrove species to be used for synthesis of nanoparticles were *Rhizophora mucronata* and *Rhizophora apiculata* by their leaves extract using the bioreduction method. The size of synthesized nanoparticles was 20–100 nm. Synthesis of gold nanoparticles was also reported using the polysaccharide-mediated method by the whole plant of *Padina gymnospora* (a mangrove associates) and synthesized NPs ranged between 53 and 67 nm (Gouda et al. 2015). The antimicrobial potential of synthesized silver nanoparticles (AgNPs) from mangroves against some bacteria is also reported in research papers. AgNPs synthesized using leaves extract of *Ipomoea pes-caprae* show antibacterial activity against *E. coli, K. Pneumonia, P. Aeruginoa, Enterobacter* sp., and *S. aureus* (Subha et al. 2015; Veeramani et al. 2018; Satyavani et al. 2013).

Photo-mediated green synthesized AgNPs using two mangrove species *Heritiera fomes* and *Sonneratia apetala* possess antimicrobial activities with sizes of 20–30 nm and 70–100 nm, respectively (Thatoi et al. 2016). The leaf extract of *Excoecaria agallocha* mangrove is also utilized for silver nanoparticles synthesis (Sangeethaarun et al. 2014). Furthermore, *Acanthus ilicifolius* mangrove plant-synthesized silver nanoparticles confirm the larvicidal activity against *Armigeressubalbatus* and *Aedesaegypti* mosquito (Ali et al. 2015). Biosynthesized silver nanoparticles (71–110 nm) using mangrove plant *Avicennia marina* extract possess a higher antimicrobial activity against gram-negative and gram-positive bacteria (Gnanadesigan et al. 2012). Researchers also demonstrated the mosquito larvicidal activity of *Avicennia marina* mangrove species.

Moreover, the mangrove associate *Hibiscus tilliaceus* plant leaf extract was evaluated for insecticidal activities against *Spodoptera litura* and *Helicoverpa armigera* and also antibacterial activity against *Xanthomonas campestris* and *Ralstonia solanacearum* (Rani et al. 2016). Synthesized silver nanoparticles showed an average size of 75 nm.

4 Conclusion

Nanotechnology is an emerging science that deals with nanomaterials ranging from 1 to 100 nm. The nanomateials have high surface-to-volume ratio which makes it applicable to various fields such as health care, food industry, and medical devices. Syntheses of these nanoparticles using physical and chemical methods are harmful for the environment as well as for humans. Thus, plant-mediated green synthesis of nanoparticles is a widely used method by researchers, as it is nontoxic, ecofriendly, cost-effective, and can be used for large-scale production. Mangroves are exclusively utilized for synthesis of nanoparticles due to their unique properties to survive in extreme conditions and also mangrove-synthesized nanoparticles have different properties from those of terrestrial plants. Nowadays, green synthesized nanoparticles have increased application in biomedical technology because of their unique properties, and this encourages researchers to study and advance further in this field.

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Chapter 16
Nanoemulsion Formulation as an Effective Therapeutic Drug Delivery System in Diabetes Mellitus

Ashwini Devaraj and Gayathri Mahalingam

Contents

1 Introduction

From ancient times, research involves the discovery of various new medicines, compounds, metabolites, and related pharmaceutical products. The findings like new compounds, metabolites and research data related to their discoveries were proceeded further as a potential therapeutic agents, and these materialized as conventional method of drug delivery (Langer 1990). The introduction of therapeutic agents into the human body and their mechanisms are known as the conventional drug delivery systems. Before the existence of drugs as delivery agents, the grinding of medicinal plants, leaves, or roots and the inhaling of smoke of the burning medicinal herbs were followed as the drug delivery in olden days (Nikam et al. 2018). In the eighteenth and nineteenth centuries, the conventional way of drug delivery system underwent a modification, which included the uniformity and texture of the drug to reach the target, mainly as capsules, tablets, eye drops, creams, or lotions, and the endovenous method of delivery. These are the major conventional forms of drugs delivered into the human body as remedial methods for the diseases. But the security and efficacy of the drugs would not reach the target area because of the lack

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of permeability. The traditional drug delivery system had many drawbacks like missing chances of drug dosage whose half life is less and its frequency of administration is more. Also, attaining the steady state condition is less. then, more flucctuation leads to high dosage or inadequate dosage leads to adverse effect, which were overcome by the development of the new advanced methods of drug delivery.

The new mode of advanced drug delivery system involves the evolution of the nanotechnological approach (Nikam et al. 2018). The new drug delivering mode is enhanced by the means of restrained ratio, passive distribution, focused delivery, and with these concepts including some other devices and techniques, a new technology has emerged and is called the "controlled release technology" (CRT). These CRTs are being popularized by many researchers and publications, and transformation has happened in the treatment and remedy of a lot of diseases with these CRT systems. The transdermal delivery, transmucosal approach, nasal inhalation, and buccal delivery of drugs are the few ways in which the modified drug delivery CRT systems function (Tiwari et al. 2012). Nanotechnology is the emerging advanced scientific field that includes various branches such as chemistry, physics, and biology, and it also unfolds the novel nanodimension structures involving effective therapeutic application for pharmacology and biomedical field.

2 Nanoemulsion and Drug Delivery

The drug delivery system has advanced from microscale to nanoscale fairly. The perfect drug delivery system is capable of performing the focused drug release and the control over release of the pills. The focused spot release will ensure enhanced efficacy of the drug molecule with fewer side effects. The control mechanism over the drug dispensation will reduce the side effects of the drug release and target. The construction and standardization of the nanoscale system for the drug delivery are drawing attention of many researchers and scientists for many reasons (Simonazzi et al. 2018). The nanodimension has various properties like optical, magnetic, and structural surface area ratio, which make it an interesting field of research in all aspects. Especially as the surface area is high in nanoscalar drugs or devices, it is utilized as a nanocarrier and a nanoadsorbent, and acts as a nanocarrier of drugs, proteins, or probes. Figure 16.1 shows the structure of nanoemulsion.

One such nanoscalar approach in the drug delivery system is the formulation of nanoemulsions. Nanoemulsions are very stable in kinetics, and it is the nanosized emulsion that contains oil-in-water (o/w) droplets of small size ranging from 50 to 200 nm (Ledet et al. 2014). Nanoemulsions have attractive dimensions such as easy preparation, thermodynamic stability, elevated surface area, transparency in the optical property, and identical small droplets (Lovelyn and Attama 2011) and thus the pharmaceutical compounds with less bioavailability will get maximum bioavailability with the help of nanoemulsion development. (Hassan and Mujtaba 2017).

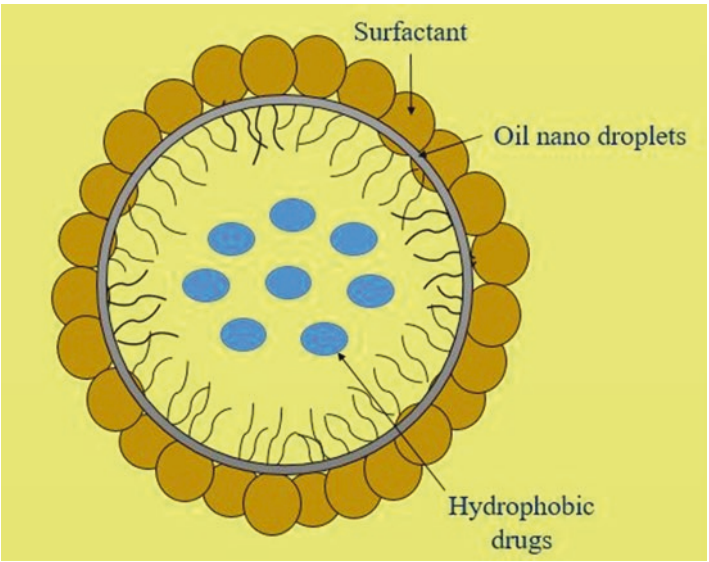

Fig. 16.1 Nanoemulsion – the capturing of hydrophobic drugs for passive release

The dispersion of one phase to another phase as minute droplets is generally called nanoemulsion; as shown in Fig. 15.1, there are two different phases with the drug trapped inside.

3 Nanoemulsion, Its Preparation, and Applications as Drug Delivery

Nanoemulsions based on their composition are classified into three types: they include oil-in-water dispersion (O/W nanoemulsions), water-in-oil dispersion (W/O) nanoemulsions, and bi-continuous nanoemulsions. Nanoemulsions come under the multiphase colloidal system and are known for their stability and translucent nature without high energy expenditure (Savardekar and Bajaj 2016). Few of the methods are listed below through which nanoemulsions are made. They are

1. High-pressure homogenization
2. Ultrasonication
3. Micro-fluidization
4. Phase inversion method
5. Hydrogel method
6. Solvent evaporation technique
7. Spontaneous emulsification

Table 16.1 Different approaches of nanoemulsion and specific disease condition for the drug transmission

S. No.	Way of drug delivery approach	Disease condition
1	Parenteral nanoemulsions	Cancer
2	Topical nanoemulsions	Psoriasis
3	Intra nasal drug delivery	Alzheimer's, Parkinsonism, psychotic drugs for targeting
4	Transdermal drug delivery	Diabetes
5	Oral route of drug delivery	Diabetes, AIDS
6	Vaccine development	Immunization vaccines
7	Otic and ocular drug delivery system	Retinal neovascularization
8	Pulmonary route	Pulmonary aspergillosis

In the present review, the nanoemulsion acts as a drug transmission system for the managing and controlling of diabetes and has different routes of applications such as parenteral nanoemulsion, drug delivery through the mouth, topical approach, transdermal drug delivery, intra-nasal drug delivery, otic and optic drug delivery system, and for cancer therapy, cosmetics, vaccine development (Chime et al. 2014), bioterrorism, and also in cell culture technology (Singh et al. 2017). Different approaches of nanoemulsion and specific disease conditions for the drug transmission are presented in Table 16.1.

4 Diabetes Mellitus (DM)

Diabetes is a lifestyle disorder, which could be managed well by altering the diet intake with regular physical activities and maintaining balanced weight. Diabetes mellitus is a silent killer because the uncontrolled increase in blood glucose leads to many long-term complications that ultimately lead to a fatal condition without showing any prior symptoms. The dangerous elements of DM are shown in Fig. 16.2. The pancreas performs a key role in the controlling of high blood glucose level.

The condition when there is high glucose in the blood is termed as hyperglycaemia, as shown in Fig. 16.3a and various organ involvements are shown in Fig. 16.3b; this is because of lack of absorption of glucose in the cells due to the impairment of pancreas, which leads to insulin resistance and causes type 2 DM. The islets of Langerhans in the β cells secrete the insulin if the hyperglycaemic condition exists, which in turn orders the liver to store the excess glucose as glucagon. Thus, the β-cells dysfunction, resistance to insulin, and persistent inflammation are the major pathophysiological conditions. The healthy functioning and unhealthy functioning of pancreas lead to insulin secretion and its lacking leads to type 2 DM as shown in Fig. 16.4a, b.

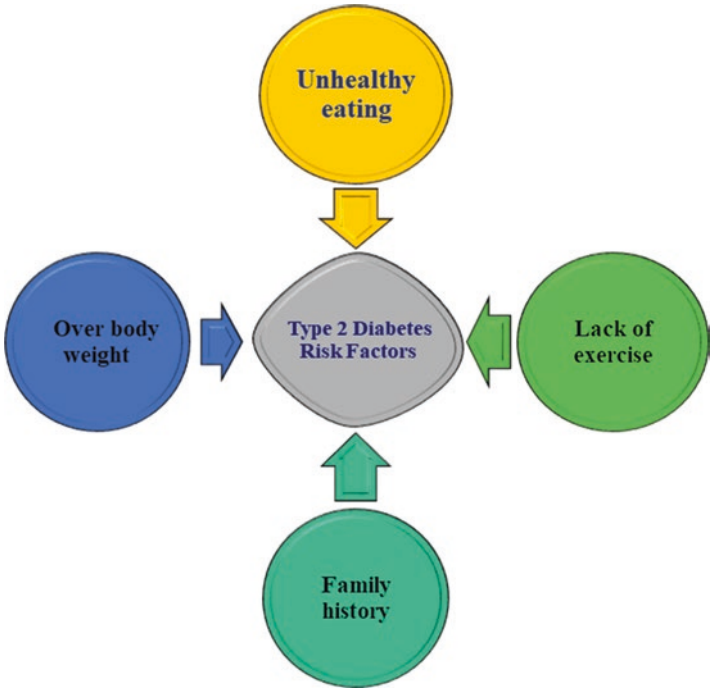

Fig. 16.2 Major risk factors of type 2 diabetes mellitus

As per the International Diabetes Federation (IDF), the prevalence of diabetes globally in the age group 20–79 years in 2017 is 424.9 million (346.4–545.4 million) and it is predicted to rise to about 628.6 million (477.0–808.7 million) in 2045 (IDF Diabetes Atlas 2017). The environment and genetics both play an important role in the pathophysiology of diabetes for an individual; hence, it is said to be a multifactorial diseased condition (Fitipaldi et al. 2018; Tiwari 2015). Insulin plays a major role in the management of diabetes by injection and includes much discomfort to patients. This will be rectified by the intervention of nanotechnology in the administration of insulin by various modes of administration such as transdermal, nasal, pulmonary and closed-loop delivery systems (Tiwari 2015). According to WHO, the management of diabetes is mainly correlated with healthcare sectors and easy access to medicines and technology and patient's active participation (WHO 2016 2016). The patient's participation is a big question mark due to cost of medications and insulin injections. These can be rectified by the involvement of nanoscale devices and drugs with targeted drug delivery in a cost-effective manner.

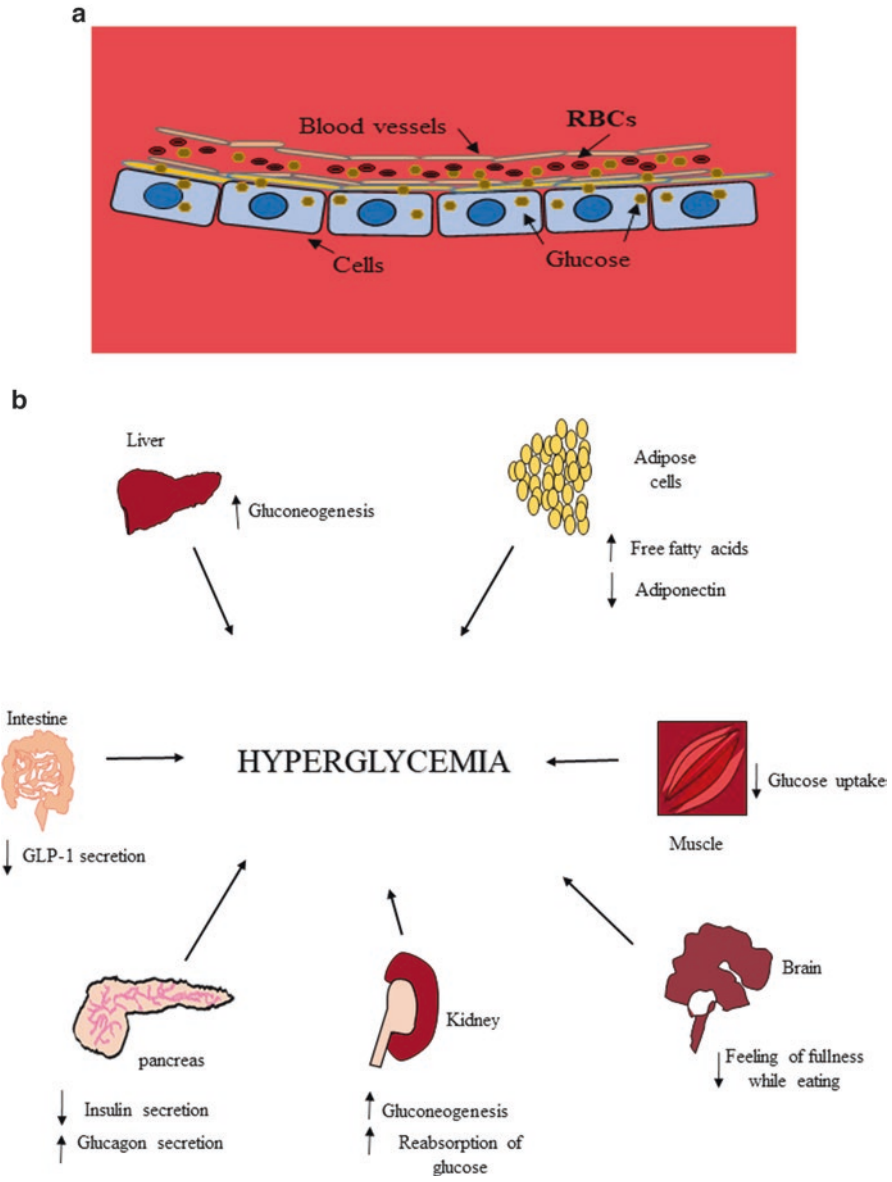

Fig. 16.3 (**a**) The hyperglycaemic condition. (**b**) Factors associated with the hyperglycaemic condition

5 Applications of NE as Therapeutics in DM

In DM, the most common method of management is the insulin dose either by injection or orally; the advancement in insulin therapy is the involvement of nanotechnology, which will be either nanoparticle or nanoemulsion systems. The nanoscale

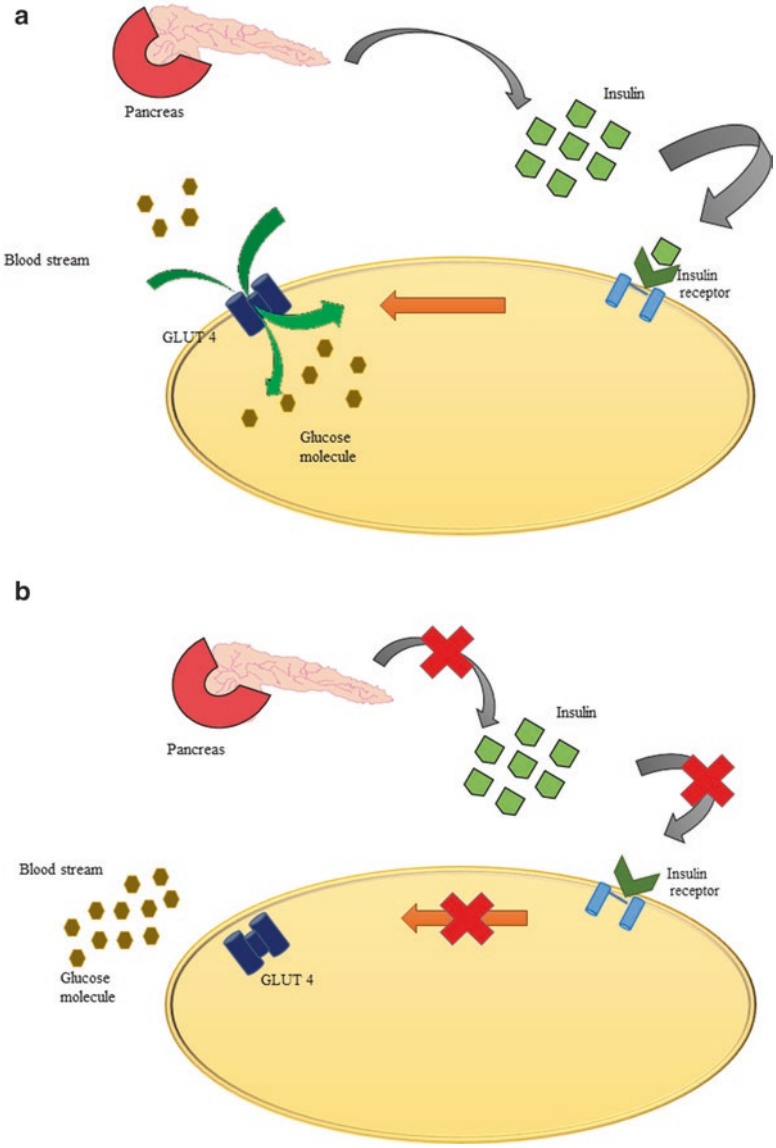

Fig. 16.4 (**a**) Healthy functioning of pancreas and glucose monitoring. (**b**) Type 2 diabetic mellitus condition and insulin deficiency

approach in DM is mainly done by using the transdermal delivery mode of insulin therapy. The formulations based on the nanoscale level will gain a greater approach in the management of DM among scientists and researchers (Veiseh et al. 2015). Since diabetes is multifactorial from both the genetic and non-genetic base, it is associated with many long-term complications; so, the research and scientific

community is focused towards using the nano-based formulations as the drug delivery system (Woldu and Lenjisa 2017) for complications associated with diabetes.

The transdermal way of nanoscale formulation is the most convenient method of drug delivery compared to oral dosage. Because the oral dosage has to pass through the gastrointestinal tract and liver enzymes and is digested or lost due to bioavailability, in order to reduce the risk of loss of bioavailability, predominantly transdermal drug delivery is preferred (Li and Gupta 2019). Thus, the dosage is minimized and bioavailability is increased with the nanoscale approach of drug delivery since diabetes is a long-term medication system. The various nanoemulsion formulations using medicinal plants and other modes, its preparation methods and mode of application are listed in Table 16.2.

Table 16.2 Various nanoemulsion formulations, their methods of preparation and applications as a drug delivery agent in diabetes mellitus

S. No.	Nanoemulsion	Method of preparation	Application in drug delivery	References
1	Nano formulation of fenugreek oil	Emulsion phase inversion and emulsion titration technique	Potent anti-diabetic properties	Hassan and Mujtaba (2017)
2	Microcapsules possess replacement islets of Langerhans cells	Microcapsule containing pores	Glucose control feedback loop	Tiwari (2015)
3	2,4,6-triphenylaniline (TPA), olive oil, surfactant (tween 80)	Coarse emulsion	Oral lipid-based drug delivery for diabetes	Ranganathan and Mahalingam (2019)
4	Pioglitazone (PZ) encapsulation	Carbopol-based transgel	Transdermal drug delivery for diabetes	Prasad et al. (2016)
5	Bay leaves (*Eugenia polyantha Wight*) ethyl acetate fraction	Tween 80: PEG 400: Virgin coconut oil (30%: 60%: 10%) in 5 mL	Self-nanoemulsifying drug delivery system (SNEDDS) for the treatment of antidiabetic mellitus type-2 resistance insulin (ADMRI)	Prihapsara (2017)
6	Bitter gourd seed oil nanoemulsion (BGO-NE)	Bioactive lipid – conjugated linolenic acid (CLNA)	Potent nutraceutical against diabetes mellitus	Paul et al. (2014)
7	Encapsulated into the Nano capsules	Tripolyphosphate sodium (TPP)	Micro-gel system, in vivo insulin delivery	Yu et al. (2016)
8	Stimuli-responsive anti-diabetic drug delivery systems	Glucose-responsive based closed-loop systems	Therapeutic potency for diabetes treatment	Yu et al. (2016)
9	Ethanolic extract of *Enicostemma littorale* (NEL)	Cross-linking with calcium chloride and solvent removal	Anti-diabetic activity in streptozotocin-induced male rats	Deepa et al. (2012)

(continued)

Table 16.2 (continued)

S. No.	Nanoemulsion	Method of preparation	Application in drug delivery	References
10	Repaglinide (RPG) nanoemulsion	Sefsol-218 (5% v/v) as an oil phase, 30% v/v of Tween-80 and transcutol as a surfactant and co-surfactant by titration method	Possess hypoglycaemic effect when compared to tablet formulation in streptozotocin (STZ)-induced diabetic experimental rats	Akhtar et al. (2016)
11	Carvacrol-based nanoemulsion	Oil/water emulsion technique, tween 80 (surfactant)	Hyperglycaemia and neurodegenerative diseases	Hussein et al. (2017)
12	Nanoemulsion of *Foeniculum vulgar*e mill. Essential oil	Oleic acid as the oil phase, surfactant tween 20 and co-surfactant propylene glycol	Transdermal nanoemulsion delivery as antidiabetic therapy	Mostafa et al. (2015)
13	(i) GCL transdermal nanoemulsion gel (ii) Nanoemulsion of essential oil of fennel	Surfactant (labrafac) and co-surfactant (triacetin) carrying Oleic acid and PG (propylene glycol; co-surfactant) as enhancer for permeation	Transdermal delivery Transdermal route	Rai et al. (2018)
14	LDL-like nanoemulsion	Plasma kinetics of both free(FC) and esterified cholesterol (EC)	Type 2 diabetes mellitus	Oliveira et al. (2009)
15	Nanoemulgel exhibited	Nanoemulsion with hydrogel matrix, emulsifying method	Therapeutic agents.	Chellapa et al. (2015)
16	Alginate/chitosan	Alg/chit through electrostatic cross-linking	Oral insulin delivery systems	Xiaoyang Li et al. (2013)
17	Magnetically responsive nanoemulsions	Intermolecular hydrogen bonding with sodium dodecyl sulphate (SDS) molecules at the oil–water interface gives stretched lamellae-like structure	Non-enzymatic approach for glucose detection	Mahendran and Philip (2014)
18	Polyethylene glycol template-assisted cupric oxide (CuO) nanoleaves on the gold-coated glass	Hydrothermal growth method	Glucose sensor electrode	Ibupoto et al. (2013)

(continued)

Table 16.2 (continued)

S. No.	Nanoemulsion	Method of preparation	Application in drug delivery	References
19	Nanoemulsion (NE) formulation of α-tocopherol (α-TC)	Mechanochemical method	Efficacious to improve the oral bioavailability and antioxidative activities in streptozotocin-induced diabetic rats	Hatanaka et al. (2010)
20	Ne containing lapachol	Emulsion phase inversion (EPI) method	Promising biological activities	Rodrigues et al. (2018)
21	Micro-needle-based delivery	Biosensors using transdermal delivery	Transdermal patch formulation for diabetes therapy	Li and Gupta (2019)
22	Thermodynamically stable (TDS) and meta- stable (MS) nanoemulsion	Lipidic artificial lipoprotein-like nano sphere	Chemotherapeutic application	Sarker (2005)
23	Insulin	Cremophor RH40 as surfactant Homogenization in a tube rotator	Enhancing oral absorption and efficacy in diabetes	Li et al. (2014)

6 Conclusion and Challenges

The fate of nanoemulsion after entering into the gastrointestinal(GI) tract when it is administered orally was discussed here (Singh et al., 2017). The lipases were one of the digestive enzymes which will digest the lipid layer of nanoemulsion, thus forming the simple lipid forms of diglycerides and fatty acids, and oil droplets. Further the digested simple lipid forms were simplified in to smaller units in order to reveal the drug into the biological system. But it has to undergo a further process by bile and other mechanisms which leads to the diffusion or transcellular mechanism of drug molecule to perform the site-specific action.

The fate of nanoemulsion through the intravenous route will be that it enters the blood stream, and if the drug gets solubilized its outreach is far and broader to various organs; it may enter the liver, spleen, or gall bladder and also to the cellular level by undergoing phagocytosis, pinocytosis, and endocytic mechanisms. At the end, the NE will be excreted by the liver, kidney, or bilary disposals, depending upon their size and features present on their surface. Even though this review has discussed the great potentialities of nanoemulsions as a therapeutic drug delivery system, challenges are still there to be overcome in the near future. Since diabetes is multifactorial, the management of the disease condition is still a big black hole. In the pharmaceutical market, the NE has yet to get the mainstream line in order to reach the laboratory work to become the patient-friendly mode (Woldu and Lenjisa

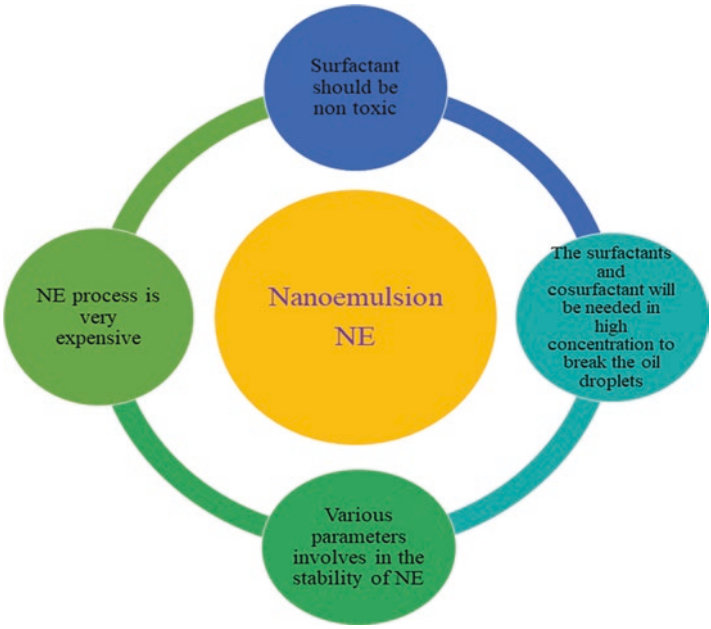

Fig. 16.5 Limitations of nanoemulsions

2017). The principal disadvantages and limitations are listed in Fig. 16.5. By overcoming all these limitations, a bright future for the nanoemulsion as a therapeutic agent in diabetes mellitus is envisaged. The nano-based drug delivery will come in common practice within next 5–8 years with all satisfactory outcomes for the betterment of human society to increase the life span by a few more years.

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Chapter 17
Synthesis of Pigment-Mediated Nanoparticles and Its Pharmacological Applications

Chidambaram Kulandaisamy Venil, Ponnuswamy Renuka Devi, and Laurent Dufossé

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1 Introduction

The demand for natural colorants is on the rise due to the detrimental effects of synthetic colors, and natural colorants have become the important part of any commodity. The major sources of natural colorants are from plants and microbes. Plant pigments have many downsides like nonavailability and pigment stability. Microbes provide an alternative source of natural colorants (Arulselvi et al. 2014). Microbial pigments have various applications and are advantageous compared to plant pigments: independent of weather conditions, low cost medium, easy processing (Manikprabhu and Lingappa 2013a). The lack of awareness and cost-effective extraction of natural ingredients hamper the improvement of natural food colorants. There is an expanded push to lessen the production costs for microbial colors by utilizing low-cost substrates or strain improvements, and soon, there might be an imposing business model market for microbial shades (Rao et al. 2017).

Nanotechnology plays an important role and is used in biotech, pharma, electronics and communication, and automobiles, etc. (Fig. 17.1) (Venil et al. 2013), and microbes have been considered for their ability for nanoparticle synthesis. Silver nanoparticles were investigated for the first time from bacteria, *Pseudomonas stutzeri* AG259, and their prospective as organic metal composites in thin film and surface coating technology was studied (Klaus et al. 1999). Microbial food nanocolorants have better shelf life, stability, solubility, and can better adhere to food and feed (Jixian et al. 2017). This chapter emphasizes the possibility of microbial pigments, their benefits and challenges, and explores pharmacological applications of pigment-mediated nanoparticles.

2 Microbial Pigments

The microbial pigments are used for various industrial applications due to their rapid growth on low-cost medium and are able to produce multicolor shades. Also, they are environmentally friendly and nonhazardous compared to synthetic dyes. Recently, quite a lot of methods have been established that include the use of

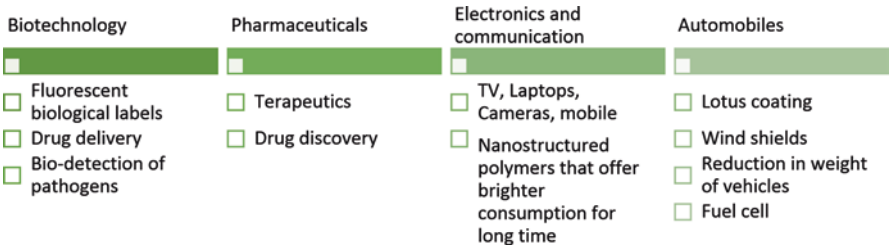

Fig. 17.1 Applications of nanoparticles

Table 17.1 Synthesis of nanoparticles by pigments

Pigment	Reaction condition	Nanoparticles	Size (nm)	Shape	Applications	References
Actinorhodin	1 mM silver nitrate + actinorhodin under sunlight	AgNPs	28–50	Irregular	Antibacterial	Manikprabhu and Lingappa (2013b)
Carotenoids	1 mM silver nitrate + carotenoids overnight at room temperature	AgNPs	65	–	Antioxidants	Fierascu et al. (2014)
Flexirubin	1 mM silver nitrate and flexirubin at room temperature	AgNPs	49	Spherical	Anticancer	Venil et al. (2016)
Fucoxanthin	2 mM silver nitrate solution + Fucoxanthin under light	AgNPs	20–25	Spherical	Antibacterial	Jena et al. (2015)
Melanin	2.5 mM silver nitrate +500 µg melanin heated at 100 °C for 10 min	AgNPs	15	–	Antibiofilm	Apte et al. (2013a)
C-phycocyanin	C-phycocyanin +1 mM $AgNO_3$ at 25 °C, pH 7, under cool white fluorescent light (50 µmol photons m^{-2} s^{-1}) for 48 h	AgNPs	–	–	–	Patel et al. (2015)
C-phycoerythrin	C-phycoerythrin +0.25 mM $CdCl_2$ + 1 mM Na_2S	Cds NPs	5	Spherical	–	Mubarak Ali et al. (2012)
Blue pigment	1 ml of pigment +20 ml of 1 mM $HAuCl_4$ under microwave irradiation for 10 s	Au nanorods	25–30	Irregular	–	Manikprabhu and Lingappa (2013c)

biological materials. Among them are nanoscale bio-pigments (Table 17.1), although they have received scanty attention compared to other biological materials.

Streptomyces coelicolor KLMP33 produces blue pigment and synthesized gold nanoparticles under microwave-assisted conditions (Manikprabhu and Lingappa 2013c). Pigment (1 mL) was made to react with chloroauric acid solution (10^{-3} mol l^{-1}) and gold nanoparticles (25–30 nm) were formed at 10 s of reaction. Further, at 30 s, gold nanorods of 45 nm were synthesized and at 90 and 120 s, the size increased to 200 and 250 nm, respectively. *Yarrowia lipolytica* NCYC 789 produces melanin pigment and Nair et al. (2013) synthesized gold nanostructures which exhibited antibacterial activities. Mubarak Ali et al. (2012) reported that *Phormidium tenue* NTDM05 produces C-phycoerythrin and nanoparticles synthesized using cadmium sulfide (CdS).

3 Nanoparticle Synthesis

Recently, nanotechnology is employed to explore the darkest avenues to combat the diseases caused by drug-resistant microbes (Singh et al. 2014). Nanoparticles have exceptional characteristics such as thermal conductivity, several properties (physical and chemical), and these properties make them applicable to electronic science, textiles, medicines, etc. The biological nanoparticles have numerous prospects like cancer therapy, gene therapy, biosensors, and antibacterial agents (Li et al. 2011). Presently, researchers work on biological nanoparticles, which are environmentally friendly, and these nanomaterials can be synthesized from plants and microbes (Fig. 17.2).

The use of sodium bromohydride in nanoparticle synthesis is unattractive due to the harmful nature of the chemical. Presently, there is a necessity to establish environmentally friendly procedures for synthesizing nanoparticles that do not employ toxic chemicals (Patra and Baek 2014). Toward the expansion of 'green chemistry,' researchers are focusing on microbial compounds that have drawn remarkable attention (Iravani 2011).

4 Different Methods of Metallic Nanoparticle Synthesis

Preparing nanoparticles by physical or chemical methods is not eco-friendly and requires attention. Physical methods use thermal or electrical energy, whereas chemical methods use harmful chemicals for nanoparticle synthesis. Many chemically synthesized nanoparticles are not appropriate for biological applications because of chemical contamination (Khan et al. 2019). Currently, efforts are made to improve the procedure consisting of environmentally acceptable solvent system, nontoxic reducing and capping agent, resulting in the green synthesis of nanoparticle

(Das et al. 2017). Biological reducing agents such as bacteria, eukaryotic, and plant extracts are used to synthesize nanoparticles (Iravani et al. 2014).

5 Pharmacological Applications of Pigment-Mediated Metallic Nanoparticles

There is increasing interest for eco-friendly natural products following the prevalence of food-related disorders. The bio-nanotechnology researchers are focusing on designing a novel strategy for well-being and nutraceuticals (Jafari and McClements 2017; Assadpour and Jafari 2019; Huang et al. 2010).

6 Antibacterial Activities of Metallic Nanoparticles

The nanoparticles exhibit microbiocidal, microbiostatic actions and assist as prospective antimicrobial agents for various pharmaceutical applications (Nasrollahi et al. 2011). Stoimenov et al. (2002) reported that the nanoparticles showed bactericidal activity against both Gram-positive and Gram-negative bacteria. The synthesized nanoparticles are effective antibacterial agents and used in therapeutic and industrial applications (Nasrollahi et al. 2011).

Patel et al. (2015) reported that silver nanoparticles from phycocyanin pigment by *Nostoc linckia* exhibited antibacterial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa, Klebsiella pneumonia and E. coli*). Phycocyanin from *Limnothix* sp. 37-2-1 formed spherical and elongated silver nanoparticles.

Biosynthesized silver nanoparticles showed significant antibacterial effect on both classes of bacteria (El-Naggar et al. 2017). Silver nanoparticles come in contact with bacterial cell wall and deactivate the production of enzymes, disrupt cell membrane, cellular proteins necessary for adenosine tri-phosphate (ATP) synthesis or influence the bacterial DNA replication functions (Agnihotri et al. 2014). Microbial synthesized nanoparticles are effective antimicrobial agents due to their least toxicity possessing in vitro and in vivo applications (Krishnaraj et al. 2010). Silver nanoparticles have a large surface area for interaction and have a bactericidal effect compared to larger nanoparticles. These nanoparticles, apart from interacting with the surface, penetrate into the bacteria and inactivate DNA replicating ability, causing the devastation of the cell.

Among the biomolecules that have been exploited in green nanotechnology are pigments obtained from microorganisms that are appropriate for biomedical applications. Manikprabhu and Lingappa (2013a) reported that *Streptomyces* sp. produces actinorhodin pigment and the nanoparticle synthesis of this pigment showed remarkable antimicrobial activity. Also, the silver nanoparticles synthesized using

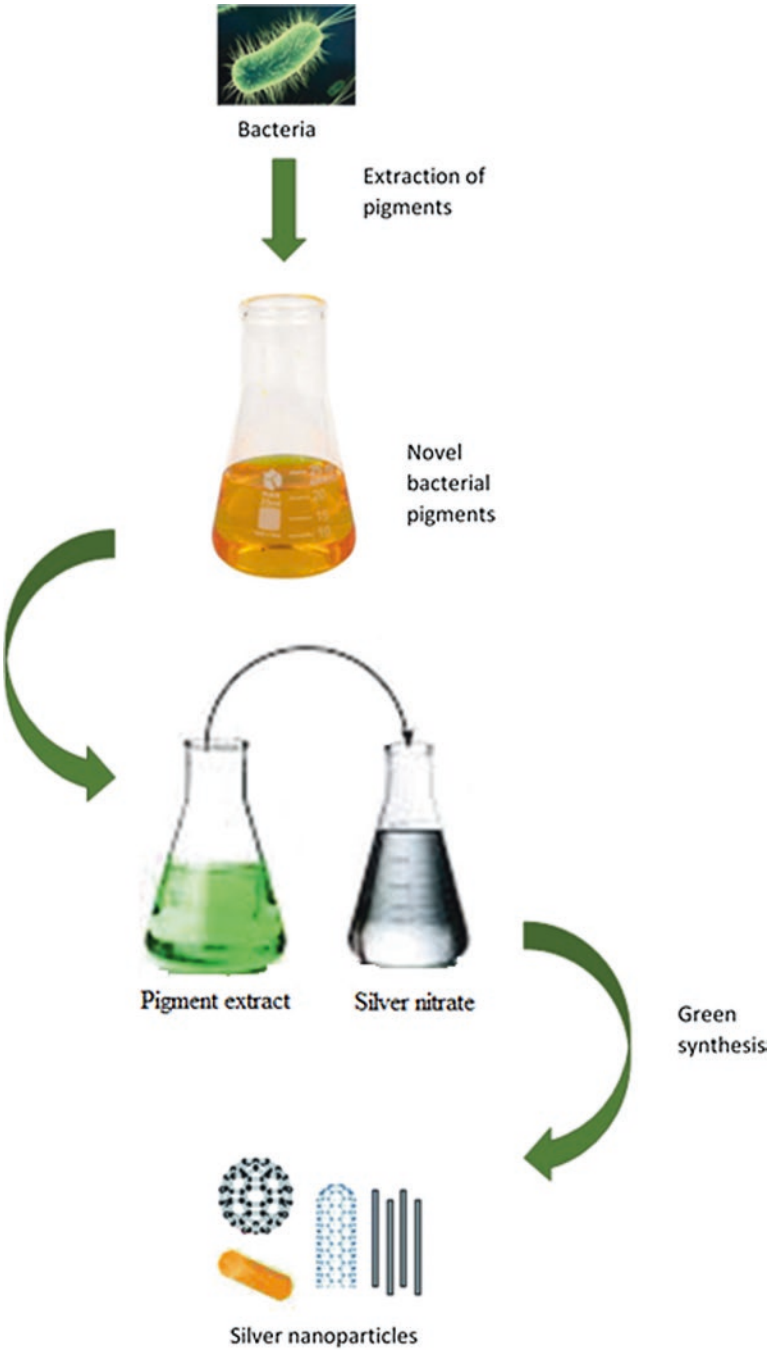

Fig. 17.2 Schematic representation for synthesis of nanoparticles using bacterial pigments

pigment from *Streptomyces coelicolor* KLMP33 showed antimicrobial activity. They display notable antimicrobial activity against extended-spectrum beta-lactamase (ESBL) producing *E. coli* (Manikprabhu and Lingappa 2014). Similarly, other microbial pigments, including flexirubin, melanin, phycocyanin, and C-phycoerythrin, showed potent antibacterial activities (Mubarak Ali et al. 2012; Apte et al. 2013b; Jena et al. 2015; Patel et al. 2015; Kumar et al. 2016;Venil et al. 2016).

7 Antifungal Activities of Metallic Nanoparticles

The synthesis and characterization of nanoparticles using natural pigments from microorganisms are still in their infancy and a lot can be learned from studies conducted on plant pigments, as some chemical families (chlorophylls, carotenoids, etc.) exist in the two sources. Bioreducing agents such as the previously mentioned chlorophylls and carotenoids are of great interest to substitute chemical and physical methods used for the synthesis of nanoparticles. Chlorophylls are pretty well known for their redox character as a basic phenomenon in the photosynthesis process. Bacteriochlorophylls *a*, *b*, *c*, c_s, *d*, *e*, *f*, and *g* produced by Purple bacteria, Heliobacteria, Green Sulfur Bacteria, Chloroflexi, etc., will be soon investigated as alternatives to plant chlorophylls, which were demonstrated to have antifungal properties against *Candida albicans* ATCC 10231 when prepared as pigment-mediated silver nanoparticles (AgNPs). Such AgNPs (50 μg/ml) were able to inhibit this unicellular fungi (26 mm identical zone of inhibition ZOI) (Baraka et al. 2017).

One interesting aspect when making pigment-mediated nanoparticles with fluorescent pigments is that you are able to follow the progress of reduction reaction, the enrollment of the pigments through easy methods such as FTIR (Fourier Transform InfraRed spectroscopy) and fluorescence analyses.

8 Anti-Inflammatory Activities of Metallic Nanoparticles

Srilekha et al. (2018) showed that a marine strain, *Micrococcus* sp., produces yellow pigment which has potential anti-inflammatory and wound-healing properties. Pigment containing ointment application resulted in effective reduction of accessory skin structures along with increase in the dermal collagen content over control.

The anti-inflammatory properties of synthesized nanoparticles were investigated by applying AgNP-coated, 0.5% silver nitrate ($AgNO_3$), or saline wound dressings to a porcine model of contact dermatitis. These coated wound dressings proved that silver nanoparticle-treated pigs had normal skin after 72 hours while other groups remained inflamed (Nadworny et al. 2008).

9 Anticancer Activity on Pigment-Mediated Nanoparticles

Because of their exclusive properties, silver nanoparticles play a significant role in the diagnosis and treatment of cancer. Jain et al. (2019) observed that the anticancer activity improved in beta-carotene solid–lipid nanoparticles (BC-SLNs) when compared to free beta-carotene. The availability of BC-SLNs is enhanced by their release from lipid core and prolongation of circulation time in the body. Solid lipid nanoparticles can be an effective and promising strategy that can be followed to improve the biopharmaceutical properties of carotenoids for anticancer effects.

10 Antioxidant Mechanisms of Pigment-Mediated Nanoparticles

Some carotenoids have pro-vitamin activity and are considered as treasured liposoluble components with antioxidant activity, which reduces the threat of infections and age-related biological transformations (Jomova and Valko 2013; Neville et al. 2013; Rao and Rao 2007; Walk et al. 2017). Carotenoids are valuable nutrients and are essential for our health and must be provided through diet (Ruiz-Sola and Rodríguez-Concepción 2012). During food processing, carotenoids are exposed to light, temperature, and in vivo conditions, that is, in the gastrointestinal tract (enzymes, acidic pH), which change the activity of carotenoids and restrict their applicability in food (Rostamabadi et al. 2019). To overcome this, encapsulation methods have been introduced for the safe passage of carotenoids into the gastrointestinal tract and their release at the targeted site (Huang et al. 2010; Jafari and McClements 2017). Among various encapsulation techniques, lipid-based nanoencapsulation is one the efficient techniques with exceptional prospects for encapsulation of carotenoids (Bhatt et al. 2016; Esposito et al. 2017; Singh et al. 2017; Xia et al. 2015).

Melanins display strong antioxidant properties, as they are negatively charged with high molecular weight formed by oxidative polymerization of phenolic compounds (Langfelder et al. 2003). The quinone of melanin via a semi-quinone state alternates between phenol and quinone (Horak and Gillette 1971). Phenolic compounds mediate synthesis of nanoparticles in biological systems (Sivaraman et al. 2009). Melanins have vast scope for applications in agriculture and pharmaceutical industries (Patil et al. 2018).

Nel et al. (2006) established the capability of melanin to act as free radicals in vitro and in vivo. Melanin acts as a nonoxidative agent and shows scavenging activities on free radicals (Bridelli et al. 2006; Perna et al. 2009). Melanin combines with oxygen, hydroxyl radical, and superoxide ion, and has been documented for its capability to prevent lipid peroxidation. Abdelhalim et al. (2018) studied the inflam-

matory liver damage in rats, and the treatment with melanin increased the alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin (TBIL), total protein, and malondialdehyde (MDA), thereby reducing the inflammation. Melanin is a strong antioxidant and can protect hepatocytes from the damage caused by oxidative stress (Patil et al. 2018).

11 Factors Influencing Synthesis of Pigment-Mediated Nanoparticles

Recently, synthesis of nanoparticles have gained utmost importance due to their unique properties and considered as the best alternative for standard drugs. The synthesis, characterization, and applications of nanoparticles are affected by several factors like size, shape, concentration, zeta potential, and researchers described the modification in nature of synthesized nanoparticles with the type of adsorbate used (Ajayan 2004; Somorjai and Park 2008). Pennycook et al. (2012) reported that synthesized nanoparticles have severe implications and change with time and environment. The properties of nanoparticles have to be given more attention in designing nanoparticles-mediated pigments for therapeutic applications.

11.1 Method

The physical, chemical, and biological methods are employed for synthesizing nanoparticles, using organic or inorganic chemicals. Among them, the biological methods, which are nontoxic and environmentally benign, are used for synthesis of nanoparticles and are more acceptable because of its green technology (Kharissova et al. 2013; Vadlapudi and Kaladhar 2014).

11.2 pH Effect

The pH plays an important role in changing the size and shape of nanoparticles. Armendariz et al. (2004) reported that the pH strongly influenced the size and structure of nanoparticles. Phanjom and Ahmed (2017) reported that alkaline conditions are mandatory for the reduction of metal ions. Moreover, the particle size also decreases with increased pH due to the silver ion reduction by electrons given by OH^- ions.

11.3 Temperature Influence

The physical methods for synthesizing nanoparticles require higher temperatures, that is, >350°C, whereas chemical methods require <350°C and biological methods require temperatures less than 100°C. The temperature of the reaction medium regulates the synthesis of nanoparticles (Rai et al. 2006). Higher temperature supports the formation of smaller nanoparticles due to the increase in adsorption of silver nitrate and reaction rate (Gurunathan et al. 2009).

11.4 Time

Mudunkotuwa et al. (2012) reported that storage time greatly influenced the characteristics of nanoparticles.

11.5 Particle Shape and Size

Akbari et al. (2011) reported that the melting point of nanoparticles is related to the size of nanoparticles and the particle size plays an important role in determining the characteristics of nanoparticles. Smaller nanoparticles will have increased stability and enhanced action compared to larger nanoparticles (Roy et al. 2019). The large surface area of smaller nanoparticles provides higher interaction area (Gurunathan et al. 2014; Raza et al. 2016). The nanoparticles of 10–15 nm have excellent antimicrobial activity (Yacam'an et al. 2001). The silver nanoparticles were synthesized using monosaccharides (glucose, galactose) and disaccharides (maltose, lactose) and tested for antimicrobial activity. The disaccharides exhibited maximum activity against Gram-positive and Gram-negative bacteria because of smaller sized nanoparticles. Li et al. (2013) showed that enhanced antibacterial effect was observed in small-sized nanoparticles of 5 nm and these small-sized nanoparticles get attached to the cell membrane easily causing membrane damage leading to cell death.

The shape of the nanoparticles also plays a significant role in antimicrobial activity due to the various degrees of interaction with the cell membrane. The antibacterial activity of different shaped silver nanoparticles (triangle, sphere, and hexagon) was studied by El-Zahry et al. (2015), and they found that hexagonal-shaped silver nanoparticles exhibited the highest activity. Few researchers reported that the shape of the nanoparticles does not have a significant effect on antimicrobial activity (Actis et al. 2015).

11.6 Concentration

The concentration of nanoparticles is another factor and is directly related to the type of microbes. Kim et al. (2007) reported that the growth of *E.coli* was inhibited at lower concentrations compared to *S. aureus.*

11.7 Zeta Potential

Zeta potential is another important factor because the interaction between nanoparticles and cell membrane is based on electrostatic adhesion (Mandal et al. 2016; Shameli et al. 2012; Phanjom and Ahmed 2017). El Badawy et al. (2011) found that there exists a direct relationship between nanoparticle surface and antimicrobial activity. Positive silver nanoparticles are susceptible to *Bacillus* strains compared to negative particles. This is because of the repulsion between negative charge on cell surface and nanoparticle surface.

11.8 Environment

Lynch et al. (2007) reported that synthesized nanoparticles formed a thick coating and were large sized when biological methods were used, and this was established by the surrounding environment.

12 Challenges in Nanotechnology

The use of nanomaterials in green chemistry is challenging in terms of toxicity to environment, health, social issues, and uncertainty in market and consumer acceptance. Due to their special properties, these nano-based products will cause risk to the ecosystem. There is a risk for nano-based products like cosmetics by interfering with cellular and subcellular mechanisms (Murthy et al. 2012). In-depth research on toxicity of nanomaterials in cosmetic formulations should be carried out with better regulations which will satisfy the consumer to choose the products. The need arises to identify the possible risks for humans and environment because of the nano-based products. Toxicity is the major problem because these nanomaterials are more reactive and toxic. When the nanoparticles enter the body, it can cause increased oxidative stress, thereby generating free radicals which lead to DNA mutation and cancer. Kampers (2008) reported that nano-based food products have been reported

for consumer safety. The specific guidelines for checking nano-based food products should be followed. The Food and Drug Administration (FDA) should develop specific guidelines for evaluating the nano-based food products for safety, packaging, etc.

13 Conclusions

Microbial pigments have demonstrated a great potential for various applications and synthesis of pigment-mediated nanoparticles has widened its scope for industrial applications. The richness of these materials in different biomolecules that can drive the process of synthesis of nanoparticles will lead to economically viable means to produce nanoparticles on a larger scale through novel green approaches. This chapter summarizes the importance of microbial pigments in nanotechnology and their pharmacological applications.

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Chapter 18
Nanotechnology and Its Role in Malaria Treatment

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Contents

1 Introduction

Malaria disease is prevalent throughout the world and is caused by the *Plasmodium* genus (an apicomplexan protozoa). Infected female *Anopheles* vector transmits five *Plasmodium* species viz. *Plasmodium falciparum*, *Plasmodium vivax, Plasmodium malariae, Plasmodium ovale* and *Plasmodium knowlesi* by biting a healthy human. Worldwide, every year, in 92 countries, an estimated 3.4 billion people are being infected with malaria and 1.1 billion people are at high risk, that is, more than 1 person in 1000 population (in these 92 countries) has the chance of getting malaria in a year. This burden was high in the African region, where 93% of death occurred due to malaria and children under 5 years contribute 61% towards malaria deaths (WHO 2018).

In the years 2017, 2016 and 2015, there were an approximated 219, 217 and 214 million malaria cases with about 435,000, 451,000 and 446,000 of death cases reported, respectively (WHO 2016, 2017, 2018).

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With a high infection and incidence rate, *Plasmodium* parasites are becoming resistant to chloroquine, sulfadoxine, pyrimethamine and arteesunate. Chloroquine is highly resistant in most malaria *P. falciparum*-affected areas. Sulfadoxine pyrimethamine (SP) has also developed resistance rapidly. In Thailand, Vietnam and Cambodia, mefloquine resistance is confined which has emerged within 6 years of careful deployment (Zuber and Takala-Harrisons 2018). In *P. vivax*, the epidemiology of resistance is less thoroughly investigated; resistance to chloroquine is pressing only in some portions of Papua New Guinea, Indonesia and nearby areas. Resistance to SP in *P. vivax* is also comprehensive (White 2004). Slowly, all *Plasmodium* parasites are gaining resistance to antimalarial drug for which combination therapy is needed. By using nanoparticles, the dilemma correlated with antimalarial drug resistance can be solved (Dennis et al. 2015).

Elimination of mosquito-breeding ground, long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS) and proper chemotherapy for patients are the four major interventions that provide for malaria reduction (Bhatt et al. 2015). Despite all efforts, malaria control is difficult because its control is also being threatened by insecticide-resistant *Anopheles* vectors (Ranson and Lissenden 2016) and inadequacy of public health insecticides (Hemingway et al. 2013). Therefore, biologically synthesized nanoparticles (NPs) have been studied for their efficacy against mosquitocidal activities to obtain the body parts of mosquito that are more sensitive because of their eco-friendly and biogenic character with a minimum dosage and organism specificity (Haldar et al. 2013).

1.1 Nanotechnology

The word ‘Nano’ means small, which comes from Greek. The study of nanosize particles that range from 1 nanometer to 100 nm in size is called ‘nanotechnology’. It is a technique that allows manipulation of properties at a very small scale. Nanotechnology concept was first proposed in 1974 by Prof. N. Taniguchi, and this field has received huge recognition since 1980. It is an essential study that is a combination of various types of NPs with different sizes, structures and various contrariety synthetic compounds (Fig. 18.1). Currently, the making of valuable metal NPs, viz. zinc, platinum, gold, silver and palladium, from biological sources has gained important attention because of an urgent necessity to produce eco-friendly technologies in these substances (Song et al. 2010).

NPs were biosynthesized utilizing biologically obtained organic compounds like proteins, carbohydrates, vitamins, lipids, biodegradable polymers, microorganisms and botanical extracts, and the possibility of development of nanotechnology has increased (Rahman et al. 2019). These improvements have affected the result of small quantity of inorganic nanoparticles, different metal oxides, metal NPs and salts. Several plant parts, viz. stem, latex, root, seed and leaf, have been recently engaged to biosynthesize metal nanoparticles. Compared to other chemicals, biosynthesis of NPs is healthy, recyclable, ecological, economical, reliable and ecofriendly (Postma et al. 1999; Rahman et al. 2019). The activity of biosynthesis is

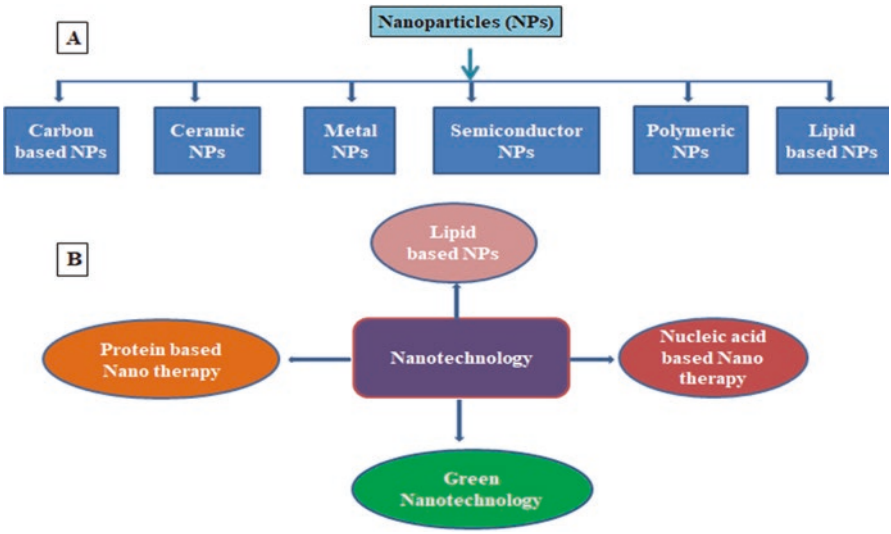

Fig. 18.1 (**a**) Different types of nanoparticles. (**b**) Different aspects of nanotechnology and its application towards malaria therapy

slower when microbes are used to biosynthesize NPs. Furthermore, nanoparticles which are restricted in size and shape are generated from microbes. Currently, fungi is considered as one of the best candidates for the biosynthesis of AgNPs (Rahman et al. 2019). Naturally occurring materials are better than artificial NPs which create ecological NPs. In this chapter, we describe the current advancement on NPs and nanotechnology for the prevention and therapy of malaria.

2 Role of Nanotechnology in Malaria Treatment

Nanotechnology fights against many parasitic diseases, viz. lymphatic filariasis, schistosomiasis, parasitic zoonoses, soil-transmitted helminthiasis, leishmaniasis ectoparasitic skin infections, onchocerciasis, tuberculosis, leprosy, and malaria. Nanotechnology works to control malaria by implementing successful therapeutic approaches to targeting parasite directly and eliminate vector. The followings are some nanotechnological applications that will assist in the simple and secure therapy of malaria.

2.1 Lipid-Based Nanoparticles

Over 20 years ago, liposome was studied to use for the therapy of leishmaniasis and malaria (Rahman et al. 2019). Liposomal formulation treatments seem superior in comparison with other malarial drug treatment. Several examples illustrate that the

toxicity of a drug is reduced if the infected tissue is targeted with a huge volume of drugs. Furthermore, the usefulness of the treatment is developed by increasing the dose instead of enhancing the dosage that is provided to the sufferers. Frequent use of nanotechnology is able to reduce the virulent property of the drug molecules (Goodsell 2004).

If artesunate liposomes with encapsulated beta artemether were released slowly for 24 hours, it reduced the dosing frequency for a malaria-resistant treatment (Chimanuka et al. 2002). Small lipid nanodrops co-loaded with artemether and lumefantrine have a greater potency and can quickly enter into the target site (Parashar et al. 2016). For meningeal malaria treatment, artemether packed into lipid NPs is an effective treatment in the animal model (Vanka et al. 2018). Experimental cerebral malaria (ECM) induced in *P. berghei* K 173-infected mice were treated successfully when different types of liposomes were used weather it coupled or encapsulated with human tumour necrosis recombinant factor, its clear form in limiting ECM-associated death by repressing mice parasite load (Rahman et al. 2019). *P. berghei*-infected mice were treated successfully when liposomes were combined with Plasmodium amino acid sequence. Peptide containing a conserved region I and consensus heparin sulphate proteoglycan binding sequence were connected to the lipid Y polyethylene glycol bio-conjugated distal end. This was fused into phosphatidylcholine liposomes (Longmuir et al. 2006).

RTS, S malaria antigen encapsulated with liposomes including lipid A, produced huge cytotoxic T-cell immune response and antibody compared to non-fusion RTS, S when it was used in mice immunization (Richards et al. 1998). In 2014, RTS, S antigen vaccine successfully prevented malaria in African children (Alonso and Noor 2017).

Polyethylene glycol (PEG)-coated halofantrine filled with poly-d,l-lactic acid (PLA) nanocapsules were examined towards malaria, and the decline in its cardio toxicity was estimated in *P. berghei*-infected mice. In the study, primaquine with diethylmethylidene malonate NPs were estimated in *P. berghei*-infected mice which shows a greater extended life span index and to target liver cell, this molecule was capsulated with gelatin and albumin NPs with different sizes (Mbela et al. 1992). Primaquine formulation with lipid nano emulsion (10–200 nm) as an antimalarial factor was found very active against *P. berghei* infection in Swiss albino mice (Singh and Vingkar 2008).

Violacein, which has anti-plasmodial (antimalarial) properties, was confirmed in vivo and in vitro (Costa et al. 2006). In the previous work, the NP violacein was examined towards *P. chabaudi* selecting mouse as a model and an accretion was found daily in the anti-plasmodial activity. However, non-infected mice obtaining equal doses of violacein did not show any notable difference in anti-plasmodial activity. Laboratory assay against *P. falciparum* suggests that violacein is more productive than the generally observed antimalarial drug quinine (Durán et al. 2007). Presently, the results of antimalarial drugs packed into immuno liposomes targeted with the compressed red blood cells (pRBC)-specific monoclonal antibody have

been investigated (Urbán et al. 2011). Antimalarial drug efficacy increased by ten fold when liposomes were encapsulated with chloroquine and fosmidomycin.

2.2 Nucleic Acid–Based Nanotherapy

MicroRNAs are noncoding RNAs which are small and engaged in gene silencing and targeting. These microRNAs are also utilized as therapeutic tools for different types of diseases, although microRNA is a physiological regulator (Rahman et al. 2014). These also play a vital function in malaria prevention. An attempt has been taken which finds that *P. falciparum* is sensitive to antisense oligonucleotide (ODN-NS) NPs (Gujjar et al. 2009). This method generally uses antisense oligo deoxy (OD) N-chitosan particles, whose size is 50 nm. These oligo deoxy particles raise the antisense ODNiinternalization by *Pf* infect erythrocytes within erythrocyte diffusion pathways that choose the *Plasmodium* topoisomerase II gene (Phillips et al. 2008). ODN chitosan NPs are +vely and −vely charged. Compared to free ODNs, ODN– chitosan NPs were observed to be higher sequence-specific inhibitors in their antisense impact. Likewise, the −vely charged surface of ODN–chitosan NPs revealed the noticeable impact of about 87% on the *P. berghei* maturity while the free ODNs revealed 68% and positively charged surface were 74%.

2.3 Protein-Based Nanotechnology for Malaria Treatment

Currently, study is continuing to produce protein-based nanoparticles for antimalarial drug distribution. Recently, Gelatin has played a major function in nano transfer system for bioactive compounds and it is utilized in pharmaceutical nanotechnology. Gelatin is a biocompatible and biodegradable collagen denatured protein where amide groups obtain it +vely charged (Yang et al. 2007; Young et al. 2005). It is also an adjuvant and sustained plasma expander because of its security report. For ideal distribution of chloroquine at a physiological pH, the gelatin NPs were used and it is taken by a double dissolution method which may then be supported by a proper cross-linking agent (Bajpai and Choubey 2006). However, adequate antimalarial activity has not been seen. Currently, to transfer malaria-specific antigens to the mark receptor favourably, nano protein adjuvants have been used. Nano protein adjuvants in combination with particular antigens varying from 16 to 73 nm in width exhibited a satisfactory immune response towards malaria-contrasted antigens individually upon injecting into the mice (Scaria et al. 2017). Due to low adaptability with target vaccine or antigen external protein, adjuvants have limited use. Additionally, Kaba et al. 2018, have designed and developed a self-assembling protein nanoparticles (SAPNs) comprising epitopes from the *P. falciparum* circumsporozoite protein (PfCSP).

Table 18.1 Impact of photosynthetic metallic nanoparticles towards *Plasmodium* parasites

Plant species name	Synthesized metallic nanoparticles	*Plasmodium* Species name
Calotropis gigantae	Titanium NPs	All sp. Of *Plasmodium* (Marimuthu et al. 2011)
Ashoka & Neem	Silver NPs	*P. falciparum* (Mishra et al. 2013)
Catharanthus roseus	Silver NPs	*P. falciparum* (Panneerselvam et al. 2011)
Andrographis paniculata	Silver NPs	*P. falciparum* (Panneerselvam et al. 2011)

NPs Nanoparticles, *P. falciparum Plasmodium falciparum*

2.4 Green Nanotechnology for the Therapy of Malaria

Gold, silver, zinc and copper are the biogenic structure of metallic NPs and have latent antimalarial actions against five *Plasmodium* species (Table 18.1). Palladium, platinum and silver are the green NPs and are proven to be more active in controlling malaria parasites. The biologically synthesized NPs are ecofriendly and have huge advantages in malarial drug production (Kuppusamy et al. 2016). Fungi, algae, yeast, bacteria and plants are used to produce carbohydrates, lipids, and proteins which are also health secure (Rahman et al. 2019).

AgNPs synthesis through plants product is eco-friendly and cost-effective (Ullah Khan et al. 2018). Leaf extracts of medicinal plants like *Garcinia mangostana* and *Acalypha indica* have been used to synthesize AgNPs of size 35 and 20–30 nm respectively (Veerasamy et al. 2011). *Capsicum annuum* and *Aloe vera* also were used for the synthesis of AgNPs (Li et al. 2007). Additionally, the leaf extract of *Euphorbia hirta* (40–50 nm) exhibited influential action, as it is a biogenic synthesized AgNPs. Like plants, both Gram-positive and Gram-negative bacteria, are being utilized for the green synthesis of AgNPs (Parikh et al. 2011).

A few bacteria also can produce extracellular AgNPs and intracellular AgNPs viz. *Aeromonas spp. SH10, Calothrix pulvinata, Vibrio alginolyticus, Plectonema boryanum UTEX 485, Lactobacillusspp and Anabaena flos-aquae* (Brayner et al. 2007; Rajeshkumar et al. 2013; Mouxing et al. 2006; Lengke et al. 2007). Current study stated that for the synthesis of AgNPs, *Bacillus licheniformis* was employed (Kalimuthu et al. 2008).

Penicillium expansum HA2N (14–25 nm) and *Aspergillus terreus* HA1N (10–18 nm) are two fungal strains also used for the synthesis of AgNPs and both these strains have outstanding antifungal potential (Ammar and El-Desouky 2016). Other studies reported that the *Aspergillus fumigatus* (5–25 nm) and *Fusarium oxysporum* (5–50 nm) are biogenic synthesized AgNPs, and had active potential towards fungal strains (Bhainsa and D'Souza 2006).

Current studies stated that red algae have also been employed for the making of AgNPs green NPs. Cellulose also plays an outstanding function in the making of AgNPs. Alcohol and Aldehyde functional group have a significant function in

cellulose modification and stabilization while Ag ion is formed (Tummalapalli et al. 2015). Likewise, other biomolecules viz. proteins and nucleic acid are also employed for the AgNPs green synthesis.

2.5 Nanotechnology for the Control of Malaria Vectors

Vector control is an essential element of malaria elimination and control strategies. Vector control has been successfully reduced or interrupts malaria transmission. By targeting various biomolecules or biochemical, physiological and molecular actions, nanotechnology controls the density of *Anopheles* vector mosquitoes (Blandin et al. 2002; Rayner 2009).

The green synthesis of nanoparticles has gained outstanding consideration because of its environment-friendly property and is effective in relation to its cost. Currently, filamentous fungus *Cochliobolus lunatic* has been utilized to synthesize metallic NPs which have strong activity against *An. stephensi* (Salunkhe et al. 2011). In India, *Aspergillus niger* has been used for the synthesis of extracellular gold NPs which is highly virulent for *Anopheles* vectors and it is also effective against *Culex quinquefasciatus* and *Aedes aegypti*. However, no after effects were observed in the environment. *Chrysosporium tropicum* is a gold and silver pathogenic fungus-mediated NPs; that it can eliminate *An. stephensi* was also reported by the same author (Soni and Prakash 2012). Numerous entomopathogenic fungi like *Trichoderma harzianum* are utilized commonly for the control of biological pests. This can be utilized to synthesize metallic NPs which can kill the *Anopheles* mosquitoes at any stage of their growth (Banu and Balasubramanian 2014). Despite fungal arbitrate NPs, plants and bacteria also maintain metallic NPs with several vital actions. Synthesized bacterial NPs of gold, cobalt, zinc, copper, and silver, by utilizing *Bacillus thuringiensis,* control the *Anopheles* mosquitoes in different regions of the earth (Marimuthu et al. 2013). The photosynthesis of NPs of several metals has also been used as an agent for controlling mosquitoes. Biosynthesized silver NPs (AgNPs) will be an expedient substitutions for synthetic chemical insecticides in future which may cause lower ecological destructions. Hence, green synthesized NPs are necessary to control mosquitoes that cause malaria (Table 18.2); (Santhoshkumar et al. 2011); however, the possibility of plant products for the biosynthesis of NPs towards *Anopheles* vector mosquitoes has yet to be completely investigated.

3 Nanotechnology and Its Limitations

Nanotechnology has an encouraging discipline to cure malaria and control the *Plasmodium* parasites as well as the vector, but no conventional principle of action of these particles has been explained yet (Foldbjerg et al. 2015). Almost all of the

Table 18.2 Impact of photosynthetic metallic nanoparticles towards *Anopheles* vector mosquitoes

Plant species	Synthesized metallic nanoparticles	*Anopheles* vector name
Cymbopogon citratus	Gold NPs	Different species of *Anopheles* vectors (Murugan et al. 2015)
Plectranthus amboinicus	Zinc NPs	*An. stephensi* (Vijayakumar et al. 2015)
Morinda citrifolia	Titanium NPs	– (Suman et al. 2015)
Nelumbo nucifera	–	Different species of *Anopheles* vectors (Santhoshkumar et al. 2011)
Eclipta prostrate	Silver NPs	*An. subpictus* (Rajakumar et al. 2011)

nanotechnological procedures correlated with the distribution of drugs; it cannot determine the exact concentration of a specific drug and has very precise impacts. However, recently, a long-lasting injectable atovaquone solid drug NPs designed by Bakshi et al. 2018, have specified applications and long-running impact. The study mainly centred on the impact of NPs towards egg, larval and pupal developmental stages of mosquitoes, but there is inadequate knowledge obtainable about the impact of these NPs on the adult mosquitoes and their ovicidal characteristics (Benelli 2016). Hence, despite an extensive investigation in this area, there are still several problems and difficulties that are required to be resolved.

4 Conclusion

Malaria control is a continuing challenge for the current study. Researchers were attempting to define an efficient procedure to cure malaria. However, due to insecticide and drug resistance, climatic, social and environmental factors, no adequate and future assuring method has been explained. From last 2 decades, the progress of superior techniques in composing liposomes, the expansion of tissue-specific nano-bio-circuits and nano-pores and introduction to green nanotechnology have provide the opportunity for a reliable, cost-effective and environment-friendly curative approach for malaria treatment. Though NPs are very effective in malaria therapy and vector control, very less research has been conducted. Industrial applications in NPs increased significantly, but till now, the relationship between NPs and biological applications are not clearly understood. Metallic NPs are widely used due to its high speed, low cost and ease of synthesis. Chemically produced NPs are restricted to use in clinical fields due to toxic materials in it. Photosynthetic metallic NPs have a significant impact on different *Plasmodium* species and *Anopheles* vector. Biosynthesized silver NPs (AgNPs) will be an expedient substitutions for synthetic chemical insecticides in future, which may cause lower ecological destructions. However, more research is required in this area to investigate the conventional principle of action and side effects.

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Chapter 19
Plant-Mediated Synthesis of Metal Oxide Nanocomposites for Environmental Remediation

Pravat Manjari Mishra

Contents

1 Introduction

Every year, a large amount of industrial effluents from the chemical, mining, textile, metallurgical industries, etc., is discharged to the environment, which is mainly responsible for contaminating the drinking water. This contaminated water contains toxic dyes, heavy metal ions such as arsenic, zinc, copper, nickel, mercury, cadmium, lead, and chromium, and other organic toxic elements that are very much harmful to the environment as well as carcinogenic to human beings.

Various methods and techniques, such as ion exchange and chemical redox followed by precipitation and reverse osmosis, have been developed for the removal of these toxic materials from both water and wastewater. But many drawbacks are involved in these processes. For example, the major drawback related to precipitation, in large-scale chemical redox method, is slurry production. Due to the high operating costs, both ion exchange and reverse osmosis methods are not economically attractive. A large number of catalysts/adsorbents have been developed for the

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removal of toxic cations and other toxic organic dyes present in both water and wastewater. But most of them have certain drawbacks, like high operational costs for treatment, high capital, and the disposal of the residual metal sludge. For example, because of high Brunauer–Emmett–Teller (BET) surface areas, activated carbon was adopted as an efficient adsorbent for the removal of the heavy metals, but it can be regenerated at a very high temperature. Thus, there is a great need to develop low-cost materials and better methods for the degradation of toxic elements present in both water and in wastewater.

Currently, the researchers are working towards the development of nanoscience and nanotechnology to provide a solution for the abatement of environmental pollutions. Due to the high BET surface areas and fast adsorption kinetics, the metal oxide nanoparticles (e.g., Al_2O_3, SiO_2, and TiO_2) and their composites are greatly considered for the treatment of water and wastewater. But the methods such as deposition precipitation, impregnation, photoreduction, and reduction, which are employed for the preparation of these catalysts, are very much expensive and involve the use of harmful chemicals such as hydrazine hydrate, sodium borohydride, dimethylformamide (DMF), and ethylene glycol that pose harmful effects to the environment. Thus, there is a great need to prepare these materials by green route without using any toxic chemicals. The use of biological materials to synthesize these nanomaterials agrees with the many principles of green chemistry, as it uses renewable materials and takes place at ambient temperatures and pressures. In comparison to other biological materials, plant extracts are very much promising for the synthesis of stable nanocomposites, as they contain a large number of phytoconstituents which are safe and easily available, and there is no requirement of growing the pure culture of microorganisms, as it is a very time-consuming and cumbersome process. The biomolecules of leaf extract, such as polyphenols, phenolics, ascorbic acid, flavonoids, and various sugars, have very high potentials to synthesize stable nanoparticles, because they act as both capping and reducing agents. The biomolecules of the plant extract formed capped nanostructures during the synthesis (Fig. 19.1). These capping agents prevent the aggregation of the nanoparticles and stabilize the nanosystem. Biocompatibility of biosynthesized nanomaterials is improved in the presence of capping agents. The use of natural surfactant in the synthesis of metal oxide may enhance the surface area, morphology, and size of the metal oxide/metal oxide composites due to the interaction of these surfactants with some specific lattice plane of metal oxide/metal oxide composites. Experimentally, it is seen that the catalysts prepared by the green method have either superior or comparable catalytic behavior in comparison to the catalytic behavior of the same catalysts prepared by the chemical methods (Arabatzis et al. 2003; Naik et al. 2013; Liang et al. 2012; Mishra et al. 2016). This method is industrially feasible and economical. But due to the availability of scanty literature in this field, more research is indeed needed to develop these highly efficient superior materials for application in environmental pollution abatement.

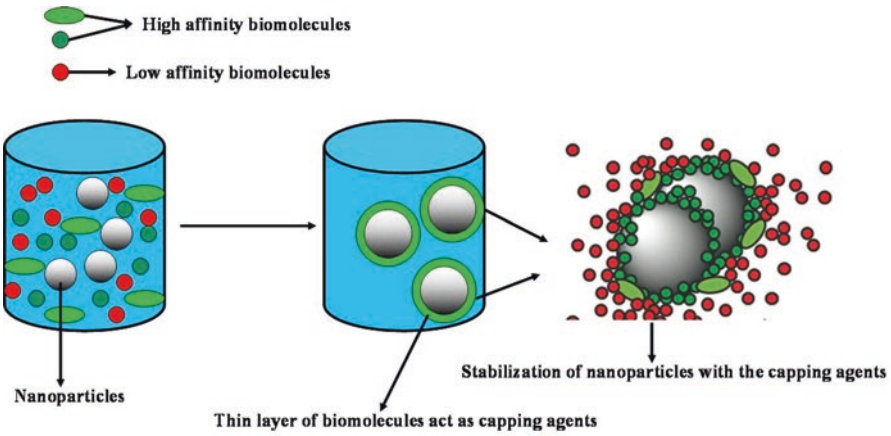

Fig. 19.1 Schematic representation of the interaction of biomolecules with the nanoparticles

2 Multifunctional Activities of Plant-Mediated Synthesized Metal Oxide Nanocomposites Towards Pollution Abatement

2.1 Degradation of Toxic Dyes

Bio-based metal oxide nanocomposites synthesized using plant biomolecules showed tremendous potential towards the degradation of toxic dyes (Table 19.1). Two-dimensional TiO_2@Ag heterojunction structure was synthesized using edible corn crispy, which exhibited efficient photocatalytic activity towards the degradation of methylene blue (MB) (Jiang et al. 2013). Liang et al. synthesized Au/TiO_2 and Ag/TiO_2 composites using *Citrus limon plant extracts, studied their photocatalytic activity for the degradation of organic dye, and reported that these catalysts are* as active as M/TiO_2 composites prepared by the chemical method (Liang et al. 2012). Naik et al. synthesized Au/TiO_2 by using aqueous leaf extract of *Cinnamomum tamala* (*C. tamala*) and also studied its remarkable activity towards the photocatalytic degradation of methyl orange (Naik et al. 2013). Cu/MgO nanocomposites synthesized by *Cassytha filiformis* L. extract showed excellent catalytic activity towards the degradation of methylene blue (Nasrollahzadeh et al. 2018a, b). Ag/RGO/Fe_3O_4 nanocomposites synthesized by *Lotus garcinii* aqueous leaf extract showed catalytic activity towards the degradation of rhodamine B (RhB) and congo red (CR) (Maham et al. 2017). Cu/Fe_3O_4 magnetic nanocatalyst synthesized using *Morinda morindoides* leaf extract showed catalytic activity towards the degradation of congo red (CR) and rhodamine B (RhB) (Nasrollahzadeh et al. 2016). (RGO)/Fe_3O_4 nanocomposites were synthesized using *Solanum trilobatum* extract and were used for the degradation of methylene blue dye (Vinothkannan et al. 2015). Ag/ZnO nanocomposite synthesized using *Valeriana officinalis L.* root extract

Table 19.1 Plant-mediated synthesis of metal oxide nanocomposites for application towards the degradation of toxic dyes

Serial number	Plant used for synthesis	Bio-based metal oxide nanocomposites synthesized	Application towards the degradation of toxic dyes	References
1	Edible corn crispy	TiO_2@Ag	Methylene blue (MB)	Jiang et al. (2013)
2	*Citrus limon plant extracts*	Au/TiO_2 and Ag/TiO_2	Orange G	Liang et al. (2012)
3	*C. tamala* leaf extract	Au/TiO_2	Methyl orange	Naik et al. (2013)
4	*Cassytha filiformis* L. extract	Cu/MgO	Methylene blue (MB)	Nasrollahzadeh et al. (2018a, b)
5	*Lotus garcinii leaf extract*	Ag/RGO/Fe_3O_4	Congo red (CR) and rhodamine B (RhB)	Maham et al. (2017)
6	*Morinda morindoides* leaf extract	Cu/Fe_3O_4	Congo red (CR) and rhodamine B (RhB)	Nasrollahzadeh et al. (2016)
7	*Solanum trilobatum* extract	(RGO)/Fe_3O_4	Methylene blue dye	Vinothkannan et al. (2015)
8	*Valeriana officinalis L.* root extract	Ag/ZnO	Methyl orange (MO), congo red (CR), and methylene blue (MB)	Yeganeh-Faal et al. (2017)
9	Pepper extract	Fe_3O_4–Pd	Acid brown and acid black	Khaghani et al. (2017)
10	Lemon extract	Fe_3O_4–CeO_2	Acid red and acid brown	Moradi et al. (2018)
11	*Picrasma quassioides* aqueous extract	GO–AgNPs	Methylene blue (MB) dye	Sreekanth et al. (2016)
12	Green tea extract	RGO–AuNPs	Congo red, safranin T, and eosin Y	Šimšíková et al. (2016)
13	*Piper pedicellatum* C.	Au–RGO	Rhodamine B, methyl red, methyl orange, methylene blue, and bromocresol green	Saikia et al. (2016)
14	*Musa balbisiana* bract extract	TiO2@C	Methylene blue (MB)	Karmakar et al. (2017)
15	*Moringa oleifera* (MO) leaves extract	Ni/Fe_3O_4	Malachite green (MG) dye	Prasad et al. (2017a, b)
16	*Euphorbia maculata* extract	Ni/Fe_3O_4	Congo red (CR), methylene blue (MB), and rhodamine B (RhB)	Pakzad et al. (2019)

(continued)

Table 19.1 (continued)

Serial number	Plant used for synthesis	Bio-based metal oxide nanocomposites synthesized	Application towards the degradation of toxic dyes	References
17	*Commersonia bartramia extract*	Cu/Al_2O_3	Congo red (CR) and methylene blue (MB)	Nasrollahzadeh et al. (2019)
18	*Commelina diffusa*	Cu/ZrO_2	Congo red (CR), nigrosin (NS), and methyl orange (MO)	Hamad et al. (2019)
19	Grape juice	$Nd_2Sn_2O_7$–Nd_2O_3	Erythrosine	Zinatloo-Ajabshir et al. (2019)
20	*Sida rhombifolia* leaf extract	Ag/ZnO	Methylene blue (MB) and malachite green (MG)	Babu and Antony (2019)
21	*Euphorbia prolifera leaf extract*	Cu/ZnO	Methylene blue (MB) and congo red (CR)	Momeni et al. (2016)

showed excellent results towards the degradation of toxic organic dyes (Yeganeh-Faal et al. 2017). Fe_3O_4–Pd nanocomposites synthesized using pepper extract had shown very good results towards the degradation of acid brown and acid black under ultraviolet (UV) irradiation (Khaghani et al. 2017). Fe_3O_4–CeO_2 nanocomposites prepared using extract of lemon showed good results towards the degradation of azo dyes (Moradi et al. 2018). GO–AgNPs synthesized through *Picrasma quassioides* aqueous extract exhibited higher activity towards the degradation of methylene blue (MB) dye (Sreekanth et al. 2016). RGO–AuNPs nanocomposites synthesized using green tea extract showed excellent results towards the degradation of toxic organic dyes like congo red, safranin T, and eosin Y (Šimšíková et al. 2016). Au–RGO nanocomposite synthesized using leaf extract of *Piper pedicellatum* C. showed very good results towards the degradation of methyl red, rhodamine B, methyl orange, bromocresol green, and methylene blue (Saikia et al. 2016). TiO2@C nanocomposites were synthesized using banana (*Musa balbisiana*) bract extract and evaluated towards the degradation of methylene blue (MB) dye (Karmakar et al. 2017). Naik et al. studied the green synthesis of Au/TiO_2 nanocomposite using *Cinnamomum tamala* leaves and its photocatalytic activity towards the degradation of methyl orange (Naik et al. 2013). Ni/Fe_3O_4 MNPs synthesized using extract of *Moringa oleifera* (MO) leaves showed very good results towards the degradation of the dye malachite green (MG) (Prasad et al. 2017a, b). Green synthesized Ni/Fe_3O_4 using *Euphorbia maculata* extract showed excellent results towards the degradation of organic dyes such as methylene blue (MB), rhodamine B (RhB), and congo red (CR) under UV irradiation (Pakzad et al. 2019). Cu/Al_2O_3 NPs synthesized using *Commersonia bartramia* extract showed great potential towards the reduction of congo red (CR), methylene blue (MB), and 2,4-dinitrophenylhydrazine

(2,4-DNPH) at room temperature in aqueous medium (Nasrollahzadeh et al. 2019). 7-hydroxy-4′-methoxy-isoflavon, extracted from the *Commelina diffusa leaf extract,* is used for the synthesis of Cu/ZrO_2 nanocomposite, which had *shown very good results towards the* reduction of various organic dyes such as congo red (CR), nigrosin (NS), and methyl orange (MO) and 2,4-dinitrophenylhydrazine (2,4-DNPH) (Hamad et al. 2019). $Nd_2Sn_2O_7$–Nd_2O_3 nanostructure, which was synthesized using grape juice, showed excellent results towards the degradation of erythrosine (Zinatloo-Ajabshir et al. 2019). Ag/ZnO synthesized using *Sida rhombifolia* leaf extract showed excellent photodegradation efficiency towards the photodegradation of methylene blue (MB) and malachite green (MG) dye (Babu and Antony 2019). Cu/ZnO NPs synthesized using the aqueous extract of the leaves of *Euphorbia prolifera showed very good results towards the degradation of* methylene blue (MB) and congo red (CR) (Momeni et al. 2016).

2.2 Degradation of Toxic Elements Present in Water Other Than Dyes

Plant-mediated synthesized metal oxide nanocomposites showed excellent catalytic activity towards the reduction of toxic elements like Cr(VI), 4-nitrophenol, 2,4-dinitrophenylhydrazine present in water. Mishra et al. studied the reduction of Cr(VI) using Au/Fe_3O_4 synthesized using aqueous leaf extract of *Averrhoa carambola* (*A. carambola*) (Mishra et al. 2016). Padhi et al. studied the degradation of phenol and reduction of Cr(VI) using green-synthesized Fe_3O_4/RGO nanocomposite (Padhi et al. 2017). Cu/MgO nanocomposites synthesized by *Cassytha filiformis* L. extract showed excellent potential towards the photocatalytic reduction of 2,4-dinitrophenylhydrazine and 4-nitrophenol in aqueous media (Nasrollahzadeh et al. 2018a, b). Ag/RGO/Fe_3O_4 nanocomposites synthesized using *Lotus garcinii* leaves showed catalytic activity towards the reduction of 4-nitrophenol (Maham et al. 2017). Pd/RGO/Fe_3O_4 nanocomposite synthesized using leaf extract of *Withania coagulans* showed very good potential towards the degradation of 4-nitrophenol (Atarod et al. 2016). RGO/ZnO hybrid nanocomposite synthesized using Prunus × yedoensis leaf extract is used for phosphate removal from aqueous solutions (Manikandan et al. 2018). RGO–Ag nanohybrid synthesized using tea polyphenols acts as an efficient catalyst in the reduction of 4-nitrophenol (4-NP) (Wang et al. 2015). Au–RGO nanocomposite synthesized using leaf extract of *Piper pedicellatum* C. showed excellent results in nitro-reduction (Saikia et al. 2016). Pd/Fe_3O_4 nanocomposite synthesized using *Hibiscus tiliaceus* L. extract showed catalytic activities towards the reduction of 4-nitrophenol (4-NP), Cr(VI), and 2,4-dinitrophenylhydrazine (2,4-DNPH) (Nasrollahzadeh et al. 2018a, b). Ag/ZnO and Ag/CuO synthesized using *Sida rhombifolia* leaf extract exhibited more significant catalytic properties towards photoreduction of 4-nitrophenol to 4-aminophenol (Babu and Antony 2019).

2.3 Biosynthesized Metal Oxide Nanocomposites as Sensors

Green-synthesized metal oxide nanocomposites also act as excellent sensors for the detection of toxic elements present in water. Ag–RGO synthesized using *Acacia nilotica* gum showed excellent sensing ability towards selective detection of Hg^{2+} ions in aqueous media in the presence of other associated ions (Gavade et al. 2019). Ag–RGO and Ag–Au–RGO synthesized using *Azadirachta indica* leaves extract were used for nonenzymatic hydrogen peroxide sensor applications (Babu et al. 2014). RGO–Au nanocomposites synthesized using rose water as reductant showed very good results towards glucose sensing applications (Tabrizi and Varkani 2014). AuNPs–RGO nanocomposite synthesized using willow bark waste was successfully used for glucose detection (Qin et al. 2013). Using the synergistic advantages of chitosan, AuNPs, and graphene nanosheets, a new plant esterase-based (PLaE–CS–AuNPs–GNs) biosensor was synthesized which was used for the ultrasensitive detection of organophosphate pesticides spiked in carrots and apples (Bao et al. 2015). Ikhsan et al. synthesized GO–Ag nanocomposite using garlic extract and applied its modified electrode as an electrochemical sensor for the detection of nitrite ions (Ikhsan et al. 2015). Biosynthesized Ag–RGO nanocomposite using *Psidium guajava* extract shows remarkable performance in detecting methylene blue with concentration as low as 10^{-8} M (Chettri et al. 2017). Al-Marri et al. studied the synthesis of graphene/Ag nanocomposites using *Pulicaria glutinosa* plant extract (PE) as reducing agent and explained that it can be used as a potential substrate for surface-enhanced Raman scattering (SERS) activities for the detection of chemical and biological analytes (Al-Marri et al. 2015). RGO–SnO_2 composite using lemon extract showed great results towards the detection of ascorbic acid (Sha and Badhulika 2018). Fe_3O_4–carbon dots prepared by lemon and grape fruit extracts are novel photoluminescence sensors for the detection of *Escherichia coli* (*E. coli)* bacteria (Ahmadian-Fard-Fini et al. 2018). RGO@AgNPs synthesized using *Pinus densiflora* leaf extracts showed excellent results towards colorimetric platform for the detection of dopamine and Cu^{2+} (Basiri et al. 2018).

2.4 Biosynthesized Metal Oxide Nanocomposites as Adsorbents

Biosynthesized metal oxide nanocomposites can be used as very good adsorbents for the adsorption of toxic elements present in water. Green-synthesized SiO_2@OPW nanocomposites prepared using orange peel waste (OPW) has shown excellent results for enhanced lead(II) removal from water (Saini et al. 2018). Prasad et al. studied the removal of toxic heavy metal ions of Pb(II) by using RGO/Fe_3O_4 nanocomposite synthesized by a green method utilizing *Murraya koenigii* (Mk) leaves (Prasad et al. 2017a, b). Ag/CuO synthesized using *Sida rhombifolia* leaf extract possesses superior adsorption capacity for MG removal (Babu and Antony

2019). RGO/Fe NPs (iron nanoparticles) synthesized using *eucalyptus* extract showed a high adsorption of MB (199.4 mg/g) (Weng et al. 2018). But scanty literature is found on the use of biosynthesized nanocomposites as adsorbents. So, more research in this field is essentially required.

3 Conclusion and Future Prospects

Exposure to water contaminated with toxic elements causes various problems related to health like severe diarrhea, epigastric pain, nausea, skin irritation, dermatitis, vomiting, and hemorrhage. Oxide-based nanomaterials (e.g., Al_2O_3, SiO_2, TiO_2 etc.) and their composites are of immense importance and have been the subjects of interest in the field of removal of organic and inorganic pollutants. Due to the hazardous effect of the chemical synthesis of these metal oxide nanocomposites, now the researchers have concentrated on the synthesis of these nanocomposites using plant biomolecules, which is eco-friendly as well as cost-effective. This chapter focused on the plant-mediated synthesis of metal oxide nanocomposites and some of their applications towards environmental remediations. It is observed that plant-mediated synthesized metal oxide nanocomposites have shown tremendous potential towards the degradation of environmental pollutants in comparison to nanocomposites prepared by chemical methods. A lot of works have been carried out on the synthesis of metal oxide nanocomposites by chemical methods. But very few works have been done on the synthesis of bio-based nanocomposites and their applications. So, it is indeed very much essential to carry out more research work on the bio-based synthesis of these novel nanocomposites by using plant biomolecules for application in environmental pollution abatement.

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Chapter 20
Actinobacterial Nanoparticles: Green Synthesis, Evaluation and Applications

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Contents

1 Introduction

Nanotechnology is a prompt, multidisciplinary emerging research area with promising applications in diverse fields such as medicine, agriculture and industry with a purpose of implementing useful materials at nano levels, with an approximate size of 0.1–1000 nm. The term nano is derived from *nanos* (Greek), which means *dwarf*. The concept of nanotechnology was put forward by the Physicist, Professor Richard P. Feynman in 1959 (Manivasagan et al. 2016). Nanoparticles are synthesised through chemical, physical and biological methods. The chemical and

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physical syntheses depend on the top-down approach, where the large-size materials are progressively crushed down to nano-ranged materials. Biological synthesis relies on the bottom-up approach that assembles the atoms or molecules to nano-sized materials. The top-down strategy is highly expensive and also generates extremely toxic compounds as by-products along with huge energy consumption. Hence, there arises a vital necessity to implement biological, environmentally friendly "green" approach for unpolluted, toxic-free, bio-compatible synthesis of technologically relevant nanomaterials (Parikh et al. 2011). The green approach utilises both plants and microbes for biological nanoparticle synthesis by exploiting the usefulness of phytochemicals and enzymes, prompting the reduction of metal compounds to nanosized particles (Narayanan et al. 2012; Chauhan et al. 2011; Hiremath et al. 2014).

Actinobacteriology is one of the emerging areas in research since actinobacteria are biotechnologically valuable and prominent group of microbes known for their inexhaustible production of biologically active compounds with applications in agricultural, medical and industrial sectors (Zotchev 2012; Velho-Pereira and Kamat 2013). Actinomycetes are gram-positive, aerobic, non-motile and filamentous terrestrial or aquatic habitants. They are unicellular, mycelium producers and share the characteristic features of both bacteria and fungi. Nearly, 10,000 bioactive metabolites have been explored from this actinobacterial group, which covered 45% of the total microbial metabolites (Anandan et al. 2016). Amongst the actinobacteria, the genus *Streptomyces* is the most economically important group since it is the producer of approximately 50–55% of recognised antibiotics (Manivasagan et al. 2014) and extensively used in pharmaceutical and enzyme industries (Alani et al. 2012).

Actinobacterial genera *Streptomyces* and *Arthrobacter* have been considered as possible 'nanofactories' and widely exploited for nanoparticle biosynthesis. In *Streptomyces* sp., more number of extracellularly synthesised nanoparticles were reported when compared to intracellular production. Gold (Ahmad et al. 2003a, b; Balagurunathan et al. 2011), silver (Sapkal and Deshmukh 2008; Alani et al. 2012), zinc and manganese nanoparticles (Chauhan et al. 2013) synthesised from *Streptomyces hygroscopicus* (Sadhasivam et al. 2012), *Streptomyces* sp. LK3 (Karthik et al. 2014), *Streptomyces viridogens* (HM10) (Balagurunathan et al. 2011), *Streptomyces naganishii* (MA7) (Shanmugasundaram et al. 2013) and *Streptomyces avidinii* (Park et al. 2006) have been reported. Nanoparticle production has also been reported in certain rare actinobacteria like *Thermomonospora* sp. (Ahmad et al. 2003a), *Nocardia farcinica* (Oza et al. 2012), *Rhodococcus* sp. (Ahmad et al. 2003b) and *Nocardiopsis* sp. MBRC-1 (Manivasagan et al. 2013a). Table 20.1 shows the actinobacterial genera utilised for nanoparticles synthesis.

Table 20.1 Different actinobacterial genera utilised for nanoparticle production

Actinobacteria	Nanoparticle
Streptomyces spp., *Thermoactinomyces* spp., *Thermomonospora* spp., *Nocardia* spp.	Gold
Streptomyces spp., *Thermoactinomyces* sp., *Nocardiopsis* sp., *Rhodococcus* spp.	Silver
Streptomyces spp.	Zinc, copper, manganese

2 Nanoparticles: Different Types

Major classifications of nanoparticle are based on molecular base and structures. Molecular-base-related nanoparticles are of two types, organic and inorganic nanoparticles. Organic nanoparticles are carbon based, which have useful applications in medicine. Inorganic nanoparticles include magnetic, noble metal and semiconductor nanoparticles. Magnetic nanoparticles (usually 5–50 nm diameter) comprise the elements with magnetic properties such as cobalt, nickel, and iron and their compounds. They can be manipulated by magnetic field and are applicable in magnetic resonance imaging (MRI), nanomaterial-based catalysts, nanofluids, imaging of magnetic particles, environmental remediation, biomedicine, optical filters and also in data storage (Lu et al. 2004; Gleich and Weizenecker 2005; Gupta and Gupta 2005). Nanoparticles based on noble metals like gold, silver, platinum, ruthenium, palladium and copper are used in biomedical applications including sensitive diagnostic assays, radiotherapy and gene- or drug delivery. They act as non-toxic carriers for gene- and drug delivery applications (Xie et al. 2010). Semiconductor nanoparticles like ZnS, ZnO and CdS are synthesised using chemical methods. This is useful in fluorescent labelling and barcodes.

Nanoparticles have three major structures, namely, liposomes, carbon nanotubes and dendrimers. Liposomes are tiny bubble, or closed-vesicle-formed phospholipid bilayers, which have hydrophilic heads pointing outwards and the hydrophobic tails pointing inwards. The inside of liposomes is water soluble and capable of protecting soluble drugs or biomolecules, and hence liposomes are used in medicine for drug delivery. They are also used in cosmetology and environmental bioremediation (Panahi et al. 2017). Carbon nanotubes are cylindrical carbon molecules with extraordinary thermal conductivity, electrical and mechanical properties. They have valuable applications in electronics, optics, nanotechnology and other areas of materials science. They are used as additives to numerous structural materials (Eatemadi et al. 2014). Dendrimers are hyperbranched-type nanoparticles and have two basic structures – one is the globular structure containing interior core where polymer branches radiate and the second has no central core and consists simply of a series of multibranched polymers. They can target the cell surface by specialised structures on the outer surface polymers called ‘molecular hooks’. They are used as nanoscale catalysts, micelle for drug- and gene delivery, chemical sensors and imaging agents (Abbasi et al. 2014).

3 Actinobacterial Nanoparticle Biosynthesis

Recently, actinobacteria have been renowned as the effectual synthesisers of nanoparticle (both extra- and intracellularly) with better size, exquisite morphology and surface characteristics exhibiting ranges of bioproperties. Compared to other fungi and bacteria, the actinomycetes secrete more proteins that consecutively raise biosynthesis production. Actinobacterial nanoparticle synthesis is a biogenic

process in which no toxic chemicals are involved (Singh et al. 2014). The elementary principle behind nanoparticle synthesis is reduction of the metal ions to stable nanoparticles by actinobacterial enzymes when provided with metal ions as substrates. Usually, the substrates provided for the secreting enzymes to silver nanoparticle synthesis are the solution of silver nitrate ($AgNO_3$) (Renganathan et al. 2013) and for gold nanoparticle synthesis, the substrates are the solution of chloroauric acid ($AuCl_4$). Other metals, such as zinc, copper and manganese, are also used for nanoparticle synthesis. Toxic heavy metal resistance to actinobacteria is mainly performed by chemical detoxification or adenosine-triphosphate (ATP)-dependent ion efflux by chemiosmotic cation (ATPase) or proton anti-transporters (Bruins et al. 2000; Beveridge et al. 1996). These processes can be carried out by intracellular bioaccumulation and extracellular biomineralisation, precipitation and biosorption. Extracellular practice of actinobacterial nanoparticle production includes fermentation, filtration of broth and complex formation of enzyme substrates in dark condition, whereas the intracellular production requires some additional steps such as treatment of enzyme–substrate complex with ultrasound or detergents. Extracellular synthesis gives commercial advantage of particular dimension with more polydispersity than the intracellular synthesis (Manivasagan et al. 2016).

3.1 Extracellular Synthesis of Actinobacterial Nanoparticles

Extracellular metal nanoparticle synthesis by actinobacteria depends on the localisation of the reductive components in the cell. It involves soluble secreted enzymes or cell wall reductive enzymes which could recognise the metal ions and get reduced to nanoparticle forms (Mohanpuria et al. 2008). The polydispersity characterisation of extracellular nanoparticles provides broad applications in electronics, bioimaging, optoelectronics and sensor technology. Extracellular nanoparticle production by actinomycetes has been widely reported. *Thermomonospora* sp., an alkalothermophilic actinomycete, produced gold nanoparticles (AuNPs) of 8 nm in size using gold chloride as the substrate (Ahmad et al. 2003a, b). AuNPs with the size range of 9–10 nm were synthesised from *Thermomonospora* sp. isolated from compost samples in the Barabanki district, Uttar Pradesh, India (Sastry et al. 2003). Extracellular AgNPs with 68.33 nm size have been produced from a soil isolate *Streptomyces* sp. JAR that revealed antimicrobial activity to broad spectrum of fungal and bacterial pathogens (Chauhan et al. 2013). Spherical AuNPs of 15–20 nm size have been synthesised from *Nocardia farcinica* collected from the National Collection of Industrial Microorganisms (NCIM), Pune (Oza et al. 2012). Subashini and Kannabiran (2013), reported that green-synthesised spherical, 20–70-nm-sized AgNPs from soil isolate, *Streptomyces* sp. VITBT7, displayed antimicrobial activity. Biologically synthesised, cubical shaped AuNPs (90 nm size) collected from the culture extract of *Streptomyces* sp. VITDDK3 were reported to possess anti-dermatophytic properties and also showed antifungal activities against *Microphyton gypseum* and *Trichophyton rubrum* (Vinay Gopal et al. 2013). *Streptomyces* sp. LK3 (JF710608) mediated AuNPs between 5 and 50 nm size possessed anti-malarial activity (Karthik et al. 2014). *Rhodococcus* sp., a metabolically versatile actinobacterium (Otari et al. 2012) and

Table 20.2 Actinobacterial nanoparticles synthesised using extracellular preparation

Actinobacteria	Nano-particle	Property	Applications	Reference
Thermomonospora sp.	Gold	Spherical, 9–10 nm size	ND	Sastry et al. (2003)
Thermomonospora sp.	Gold	30–60 nm size	Biosensor	Torres-Chavolla et al. (2010)
Rhodococcus sp.	Silver	Spherical, 10 nm size	ND	Otari et al. (2012)
Streptomyces sp.	Gold	5–50 nm size	Anti-malarial	Karthik et al. (2013)
Streptomyces sp.	Gold	Cubical, 90 nm size	Antifungal	Vinay Gopal et al. (2013)
Streptomyces griseus	Gold	Spherical, 50 nm	ND	Khadivi Derakhshan et al. (2012)
Streptomyces sp.	Silver	Spherical, 10–100 nm	ND	Faghri Zonooz and Salouti (2011)
Streptomyces sp.	Silver	Spherical, 21–48 nm	Antibacterial	Sivalingam et al. (2012)
Streptomyces hygroscopicus	Silver	Spherical, 20–30 nm	Antimicrobial	Sadhasivam et al. (2010)
Streptomyces albogriseolus	Silver	Spherical, 16.25 nm	Antibacterial	Samundeeswari et al. (2012)
Streptomyces sp.	Gold	Spherical, 10–30 nm	ND	Zonooz et al. (2012)
Streptomyces hygroscopicus	Gold	Spherical, 10–20 nm	Antibacterial	Sadhasivam et al. (2012)
Streptomyces albidoflavus	Silver	Spherical, 10–40 nm	Antibacterial	Shetty and Kumar (2012)
Thermoactinomyces sp.	Silver	Spherical, 20–40 nm	Antibacterial	Deepa et al. (2013)
Nocardia farcinica	Gold	Spherical, 15–20 nm	ND	Oza et al. (2012)
Streptomyces sp. VITBT7	Silver	Spherical, 20–70 nm	Antimicrobial	Subashini and Kannabiran (2013)
Streptomyces sp.	Silver	Spherical, 20–70 nm	Antibacterial	Subashini et al. (2013)
Streptomyces sp. JAR	Silver	Spherical, 68.13 nm	Antimicrobial	Chauhan et al. (2013)
Actinomycete	Silver	Spherical, 5–40 nm	Antibacterial	Sukanya et al. (2013)
Streptomyces sp. LK3	Silver	Spherical, 5 nm	Anti-parasitic	Karthik et al. (2013)
Streptomyces sp.	Silver	Spherical, 28–50 nm	Antibacterial	Manikprabhu and Lingappa (2013)
Nocardiopsis sp. MBRC-1	Silver	Spherical, 45 nm	Anticancer; Antimicrobial	Manivasagan et al. (2013a)
Streptomyces sp.	Silver	ND	Antimicrobial	Shirley et al. (2010)

ND not determined

Streptomyces glaucus 71MD, a novel actinobacterial strain (Tsibakhashvili et al. 2011), were reported to be the extracellular producers of AgNPs. Table 20.2 shows the reported details of extracellularly produced nanoparticles from actinobacteria.

3.2 Intracellular Synthesis of Actinobacterial Nanoparticles

Intracellular nanoparticle synthesis by actinobacteria requires some additional processing steps like ultrasound treatment or addition of certain detergents for efficient release to culture media. Ahmad et al. (2003b) have first described the intracellularly

Table 20.3 Actinobacterial nanoparticles synthesised using intracellular preparation

Actinobacteria	Nanoparticle	Property	Applications	Reference
Streptomyces sp.	Silver	15–25 nm	ND	Alani et al. (2012)
Streptomyces sp.	Manganese and zinc	10–20 nm	ND	Waghmare et al. (2011)
Streptomyces sp.	Gold	11–25 nm	ND	Sapkal and Deshmukh (2008)
Streptomyces viridogens	Gold	Spherical, 18–20 nm	Antibacterial	Balagurunathan et al. (2011)
Rhodococcus sp.	Gold	5–15 nm	ND	Ahmad et al. (2003b)

synthesised AuNPs (5–15 nm) with good monodispersity from alkalotolerant actinomycetes, *Rhodococcus* sp. These particles were attached on the cytosplasmic membrane in addition to the cell wall. Perhaps, they were more concentrated on the cytoplasmic membrane. The presence of enzymes on the cell wall and cytoplasmic membrane possibly reduces the metal ions to nano clumps. The intracellular nanoparticle synthesis, described mainly from bacteria and fungi, was only less reported from actinobacteria. A convenient, intracellular method of zinc and manganese nanoparticle (10–20 nm) production has been testified by reducing zinc sulphate ($ZnSO_4$) and manganese sulphate ($MnSO_4$) via *Streptomyces* sp. HBUM171191 (Waghmare et al. 2011). According to Balagurunathan et al. (2011), *Streptomyces* spp. strains (D10, ANS2, HM10 and MSU) isolated from the mountainous regions of the Himalaya were efficient for spherical and rod-shaped intracellular AuNP (18–20 nm) synthesis and also showed antibacterial activity. Alani et al. (2012), made a comparison of the intracellular manufacture of silver nanoparticles (AgNPs) from *Aspergillus fumigatus* and *Streptomyces* sp. Nanoparticle formation could be designated by changing the colourless solution to light brownish to dark brownish colour. After 24 h, the initial nanoparticle formation was faster with *A. fumigatus*, but continued up to 48 h with the *Streptomyces* resulting in higher concentrations. Transmission electron microscopic pictures exposed that *Streptomyces* sp. had finer size distribution of 15–25 nm than *A. fumigatus* (15–45 nm). The higher productivity and better narrower size dispersal of *Streptomyces* make clear its well-established use for industrial purposes. Table 20.3 provides the details of intracellularly produced nanoparticles from actinobacteria.

4 Characterisation and Evaluation of Actinobacterial Nanoparticles

Currently, many researchers are actively involved in actinobacterial nanoparticle manufacture through the green approach; hence, different types of nanomaterials are manufactured day by day, which ultimately requires more precise and reliable methods for their evaluation and characterisation. The nanoparticle formation can be detected by change in the colour of solution. Colour change from pale yellow to brown indicates the formation of silver nanoparticles, while the colour change from

pale yellow to pink specifies the formation of gold nanoparticles and from whitish yellow to yellow shows the production of zinc and manganese nanoparticles (Chauhan et al. 2013). Moreover, they are characterised based on the shape, size, dispersity and surface area (Jiang et al. 2009). Some common techniques relevant for the characterisation of nanoparticles are UV–visible (UV–vis) spectrophotometry, X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), dynamic light scattering (DLS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), energy dispersive X-ray (EDX) analysis and Fourier transform infrared spectrometer (FTIR) analysis.

UV–vis spectroscopy is a generally used technique to evaluate the reduction of metal ions. Wavelengths in a range of 200–800 nm are commonly employed for characterising 2–100-nm-sized nanoparticles (Feldheim and Colby Jr 2002). More precisely, absorption measurements of silver and gold nanoparticles were characterised by using the wavelength ranges of 500–550 and 400–450 nm, respectively (Manivasagan et al. 2016, 2014). XRD provides information on nanoparticle crystalline structure, size, lattice parameters and identification of phase. The freeze-dried and powdered forms used to penetrate X-rays at a speed of 0.02/min and the consequential diffraction can be related with the standard to derive the structural evidences (Vidhya and Balagurunathan 2013). The characterisation of the size, distribution and surface charge of liquid-suspended nanoparticles can be done using DLS (Jiang et al. 2009). FTIR spectroscopy is suitable for distinguishing the nanoparticle surface chemistry, for example, the presence of surface chemical residues and other organic functional groups such as hydroxyl and carbonyl groups (Zarina et al. 2014; Chithrani et al. 2006). Electron microscopy, SEM and TEM, is employed for morphological characterisation of nanoparticles (Schaffer et al. 2009). Surface morphology and size of metal nanoparticles can be determined using SEM. For SEM analysis, sonicated, dried powder nanoparticles are used (Dwivedi 2013). While comparing to scanning electron microscopy, TEM provides 1000-fold greater morphological resolution of nanoparticles. The ultrasonicated nanoparticles on a copper grid and coated with carbon or palladium were used for TEM analysis. EDX is used for determining the elemental configuration of metal nanoparticles (Strasser et al. 2010). The topological appearance and size can be determined by AFM. The AFM images also help analyse the porosity, roughness and fractal dimension of nanoparticles. A thin film of sonicated metal nanoparticles was used for the AFM study (Hussain et al. 2014).

5 Applications

Nowadays, nanoparticles synthesised using the green approach are being utilised in various applications, exclusively in diagnostics because of their antifungal, antibacterial, larvicidal, anticancerous, antifouling and antioxidative properties (Youns et al. 2011; Doria et al. 2012). Especially, AuNPs and AgNPs have broad-spectrum antimicrobial activity against animal and some human pathogens (Sivalingam et al. 2012; Vinay Gopal et al. 2013).

5.1 Antibacterial Properties

The antibacterial properties of metal nanoparticles is associated with cellular toxicity through the releasing reactive oxygen species (ROS) (Nel et al. 2009). Antibacterial properties of AuNPs and AgNPs are concomitant with their oxidation and liberation of ions such as Ag^{+} and Au^{3+}, giving ideal biocidal properties. Also, nanoparticles with well-developed surface area exhibited good antibacterial properties; hence, they deliver maximum contact to the environment (Krutyakov et al. 2008). Metal nanoparticles have shown excellent antibacterial properties by disturbing cellular permeability as well as cellular respiration. The positively charged metal ions pierce into the bacterial cell by binding and disrupting the negatively charged bacterial cell wall, resulting in the denaturation of proteins, disrupting DNA replication and ultimately leading to the death of organism (Lin et al. 1998; Morones et al. 2005). Silver nanoparticles cause the exhaustion of intracellular ATP by bursting the plasma membrane or by obstructing the respiration by changing the cell wall oxygen and sulphydryl (–S–H) groups to R–S–S–R groups, thereby leading to cell death (Lok et al. 2007; Kumar et al. 2004). AuNPs synthesised from *S. viridogens* displayed excellent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Balagurunathan et al. 2011). AgNPs from a novel *Streptomyces* sp. BDUKAS10 also showed better bactericidal activity towards certain bacteria (Sivalingam et al. 2012). According to Samundeeswari et al. (2012), AgNPs from *Streptomyces albogriseolus* helped to eliminate some food microbial pathogens, for example, *Bacillus cereus, E. coli* and *S. aureus*. AgNPs synthesised from *Streptomyces albidoflavus* through the green chemistry approach exposed the potential antibacterial effect of AgNPs against some gram-positive and gram-negative strains (Shetty and Kumar 2012). Subashini et al. (2013) reported that the AgNPs from *Streptomyces* sp. showed activity against anti-extended spectrum beta-lactamase-producing strain *Klebsiella pneumoniae* (ATCC 700603) and also against some other medically important pathogens such as *E. coli* and *Citrobacter* species.

5.2 Antifungal Properties

Recently, gold and silver nanoparticles have been materialised as potential antifungal agents. Gold nanoparticles synthesised via green approach using *Streptomyces* sp. VITDDK3 displayed good antifungal activity against *Microsporum gypseum* and *Trichophyton rubrum* by fluctuating the membrane potential and hindering the ATP synthase activity (Vinay Gopal et al. 2013). The anti-dermatophytic activity of gold nanoparticles might be due to the vulnerability to pathogen cells and the toxicity of gold metal. Nanoparticles decrease the general metabolism of pathogens by altering the membrane potential and hindering the action of ATP synthase. They also block the ribosome subunits from transfer ribonucleic aid (tRNA) binding, demonstrating a collapse of biological processes. Silver nanoparticles also improve chemotaxis in the early reaction phase (Kalishwaralal et al. 2010).

5.3 Anti-biofouling Properties

Anti-biofouling is a process of eliminating biofouling that causes microbes to aggregate on the wetted surfaces, thereby making biofilms and producing foul smell. Biofilms create certain operational problems in industrial sectors including medicine, sensor sensitivity, water treatment and shipping. The efficient exploitation of anti-fouling properties of biosynthesised nanoparticles can effectively prevent or eliminate biofilm accumulations (Chapman et al. 2010). Biosynthesised, spherical, 5–50-nm-sized silver nanoparticles from *Streptomyces naganishii* (MA7) showed effectiveness against 10 different biofouling bacteria in vitro (Shanmugasundaram et al. 2013).

5.4 Antioxidative Properties

The nanoparticles showing antioxidative properties hinder the oxidation of some molecules by obstructing the defence mechanism prominently generating ROS. Spherical, 5–50 nm silver nanoparticles synthesised from *Streptomyces naganishii* (MA7) showed potential antioxidative properties. The 1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging assay confirmed that the silver nanoparticles produced are free radical scavengers. Owing to their high effective scavenging activity, they can be efficiently used in the treatment of cancer, neurodegenerative diseases and AIDS (Shanmugasundaram et al. 2013).

5.5 Anti-parasitic Properties

Metal nanoparticles exhibited anti-parasitic properties against some parasites that transmit diseases. Nanoparticles enter into the intracellular space through larval membrane and lead to the denaturation of organelles and intracellular enzymes. Silver nanoparticles produced by *Streptomyces* sp. GRD displayed effective larvicidal activity against *Culex quinquefasciatus* and *Aedes aegypti*, which is considered as an effective bioprocessing method for mosquito control (Kaler et al. 2010). Silver nanoparticle synthesised from a marine actinobacterial isolate, *Streptomyces* sp. LK3 exhibited significant larvicidal property against *Haemaphysalis bispinosa* and *Rhipicephalus microplus* (Karthik et al. 2014).

5.6 Anti-malarial Properties

Malaria-causing protozoan parasites, *Plasmodium* species, especially *Plasmodium falciparum*, are highly resistant to available anti-malarial drugs. Chemotherapeutic strategies using the uniqueness of metal nanoparticles have been utilised as an addi-

tive to numerous drugs, which are labelled as passive medicines. An effective gold compound, Auranofin (AF), shows potential anti-malarial property by hindering the growth of *P. falciparum* (Panyala et al. 2009). The precise mechanism of action is still unknown, perhaps it acts via immunological mechanisms and by altering lysosomal enzyme activities (Sannella et al. 2008). Karthik et al. (2014), reported that the biosynthesised gold nanoparticles from *Streptomyces* sp. LK3 possessed anti-malarial activity in addition to other anti-parasitic activities and might be deliberated as an impending foundation for drug development against malaria.

5.7 Anticancerous Properties

Nanobiotechnology delivers an effective tool for diagnosing and treating cancer through the minimisation of costs and side effects. Biosynthesised silver nanoparticles by a new strain, *Nocardiopsis* sp. MBRC-1, isolated from oceanic sediments of Busan coast, South Korea, showed in vitro cytotoxic action against HeLa cell lines (the cervical cancer cell line) (Manivasagan et al. 2013a). Shanmugasundaram et al. (2013), reported that silver nanoparticles synthesised using *S. naganishii* (MA7) isolated from Salem district of Tamil Nadu in India also unveiled cytotoxic property against HeLa cancer cell lines.

5.8 Biosensing Properties

Gold nanoparticles produced using *Thermomonospora* sp. can be utilised as biosensing enhancement analytical devices meant for detection purpose in pollution control field and military field (Torres-Chavolla et al. 2010).

6 Conclusion

The application of synthesising strategies with biological processes and green chemistry led to the development of an environment-friendly approach in non-toxic nanoparticle production. In contrast to other hazardous processes, biosynthesis of nanoparticles from actinobacteria is eco-friendly and cost-effective, and the nanoparticles thus produced exhibit potential biological properties, for example, antifungal, antibacterial, anti-biofouling, anticancerous, anti-parasitic, anti-malarial, and antioxidative. Due to their rich actinobacterial diversity and innate potential for nanoparticle synthesis, actinobacteria are considered as significant ‘biofactories’ for nanoparticle manufacture. The extensive knowledge of bioprocess mechanisms, chemical structure and reaction kinetics of actinobacterial nanoparticles might lead to further applications.

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