Chapter 11
Cytotoxicity and Genotoxicity of Biogenically Synthesized Silver Nanoparticles

Nelson Durán, Amedea B. Seabra, and Renata de Lima

Abstract In recent years, the development of nanotechnology has been focused on the development of protocols to synthesize important technological and medical metallic nanoparticles, such as silver nanoparticles, based on clean, nontoxic, biocompatible, and environmentally friendly approaches. "Green" synthesis of nanoparticles can be successfully performed extracellularly or intracellularly by organisms such as bacteria, yeast, fungi, algae, and plant extracts. Only in the recent past, biogenic syntheses of metal nanoparticles have gained significant attention. Silver nanoparticles (AgNPs) are considered one of the most important and commonly used metallic nanoparticles, in particular in medical applications, due to their known antimicrobial activities. In this scenario, this chapter discusses the recent developments on the biogenic synthesis of AgNPs by bacteria, yeast, fungi, algae and plants, highlighting the advantages and drawbacks of biogenic syntheses methods. Moreover, in order to propose any biological applications of AgNPs, it is mandatory to detailed investigate the toxicity of this nanomaterial. In this context, this chapter also discusses recent progress on the in vitro and in vivo cytotoxicity and genotoxicity of biogenic and chemically synthesized AgNPs. Although important progresses have been reached in this domain, there is still a necessity of more and detailed studies on the toxicity of AgNPs, in particular on biogenic AgNPs. Therefore, this chapter hopes to be a source of inspiration for more studies on the biogenic syntheses of AgNPs and the fully characterization of their toxic effects on humans and on the environment.

N. Durán (✉)
Biological Chemistry Laboratory, Institute of Chemistry, Universidade Estadual de Campinas, CEP 13083-970, Campinas, São Paulo, Brazil

Center of Natural and Human Sciences, Universidade Federal do ABC – UFABC, Santo André, São Paulo, Brazil
e-mail: duran@iqm.unicamp.br

N. Durán et al. (eds.), *Nanotoxicology*, Nanomedicine and Nanotoxicology,
DOI 10.1007/978-1-4614-8993-1_11,

11.1 Introduction

In recent years, the accelerate expansion of nanotechnology has led to the production of various commercially available nano-sized materials, such as silver nanoparticles (AgNPs) (Ahmed et al. 2008), which received considerable attention due to their potent antimicrobial activities (Durán et al. 2010). AgNPs can be synthesized by traditional chemical methods, which are considered aggressive to the environment and to human health. Biogenic syntheses of AgNPs have been emerging as an attractive "green" alternative to synthesize nanomaterials for diverse applications (Bansal et al. 2012).

AgNPs have been used in many commercial products (Hood 2004; Wijnhoven et al. 2009) with different chemical and physical properties (Wise et al. 2010; AshaRani et al. 2008). The increased uses of AgNPs in several applications led to a concern regarding the toxicity of these nanoparticles and the safe use of these nanomaterials, as discussed in diverse subareas of nanotechnology, such as nanobiotechnology and nanomedicine (Brayner 2008; Panda et al. 2011). In fact, nanoparticles may have higher toxicity than bulk materials (Donaldson et al. 1999; Xiong et al. 2011). Nanoparticle toxicity direct impacts human health and the environment, and more investigations are needed in this topic (Nel et al. 2006; Lewinski et al. 2008; Ju-Nam and Lead 2008).

Important studies have demonstrated that size and the chemical nature of nanoparticles coating can lead to different toxic effects at the cellular, subcellular, and biomolecular levels, such as genes and proteins (Gurr et al. 2005; Chi et al. 2009). Most of the in vitro assays to characterize the toxicity of AgNPs have been carried out in different cellular models, such as human lung fibroblasts (AshaRani et al. 2009). The majority of the studies reported an increase in the oxidative stress and severe lipid peroxidation, as observed in the case of fish brain tissue upon exposure to nanomaterials (Oberdörster 2004). In general, the proposed mechanisms for these effects consider the induction of reactive oxygen species (ROS) formation by AgNPs, which is associated with DNA damage, apoptosis, and necrosis (Arora et al. 2008; Kim et al. 2009a, b; Foldbjerg et al. 2011). In vitro exposure to AgNPs showed a reduction in glutathione levels and the observation of lipid peroxidation (Arora et al. 2008; Kim et al. 2009a, b). In this scenario, complete elucidation of the toxicity mechanisms of AgNPs is of paramount importance, and more studies based on the cytotoxicity and genotoxicity of AgNPs are still necessary. In this regard, many important reviews described the methodologies currently available for in vitro and in vivo genotoxicological studies of nanomaterials, including AgNPs (Ng et al. 2010; Johnston et al. 2010; Rico et al. 2011; Gonzalez et al. 2011; de Lima et al. 2012; Doak et al. 2012). Therefore, the aim of this chapter is to present and discuss recent progress on the synthesis of AgNPs by using environmentally friendly approaches, which minimizes negative impacts of nanoparticles on human health, and also the evaluation of the toxicity of AgNPs, highlighting the necessity of more studies in this exciting area.

11.2 Biogenic Silver Nanoparticles: Preparation and Characterization

Traditional chemical methods of synthesis of metallic nanoparticles, in particular AgNPs, employ toxic reagents, release harmful byproducts to the environment, consume a lot of energy, and use expensive chemicals. Moreover, chemical syntheses result in the absorbance of toxic chemicals on the surface of nanoparticles (Bansal et al. 2012). In contrast, microorganisms and plants extracts are considered interesting nanofactories in the fabrication of metallic nanoparticles, in a novel concept of environmentally friendly synthesis of nanomaterials (Antony et al. 2011; Bai et al. 2011; Kumar et al. 2012a). In this context, the use of plant extracts and microorganisms in the synthesis of NPs has been emerged as a biocompatible, "green," and exciting approach. In fact, biogenic synthesis of NPs has been gained a tremendous attention in recent years, since it is cost-effective and environmentally friendly. Either uni- or multicellular organisms have been shown immense potential for the synthesis of NPs, since they are able to synthesize AgNPs either intracellulary or extracellulary (Birla et al. 2009). Biogenic synthesis of inorganic NPs employs mild experimental conditions, such as pH, pressure, and temperature, leading to the formation of NPs coated with capping layer formed by proteins and/or lipids that confer physiological solubility and stability for the nanoparticles (Banu et al. 2011; Fayaz et al. 2011; Li et al. 2011). In this scenario, this section highlights recent developments on the biogenic syntheses of AgNPs by bacteria, yeast, fungi, algae, and plant extracts. The most common techniques used to characterize the obtained biogenic AgNPs are UV-visible spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction, Fourier transform infrared spectroscopy (FTIR), and Raman spectroscopy (Kora et al. 2012).

11.2.1 Bacterial Synthesis

Recently, many bacterial species have been successfully used to extracellularly synthesize AgNPs (Bai et al. 2011; Bansal et al. 2012). Biogenic synthesis of AgNPs by bacteria is carried out by the incubation of silver ions with cell filtrates of bacteria at room temperature for minutes–hours.

Incubation of bacterial culture isolated of *Pseudomonas stutzeri* AG259 with silver ions led to the formation of AgNPs with controlled size and distinct morphology within the periplasmic space of the bacteria (Klaus et al. 1999; Joerger et al. 2000; Klaus-Joerger et al. 2001). Recently, marine actinomycetes strain of *Streptomyces albidoflavus* was used to synthesize either extracellular or intracellularly AgNPs (Prakasham et al. 2012). The obtained AgNPs showed high stability in aqueous solution. This stabilization could be attributed to the secretion of proteins by the bacterium in the reaction mixture. Moreover, biogenic synthesized AgNPs

revealed antimicrobial activities against both Gram-negative and Gram-positive bacterial strains (Prakasham et al. 2012). Fayaz et al. (2011) reported the preparation of stable AgNPs through the exposition of cell-free extract of the bacterium *Geobacillus stearothermophilus* to $AgNO_3$ solution. The stability of AgNPs was attributed to the presence of capping proteins on nanoparticle surface, as evidenced by FTIR and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). The formation of AgNPs was confirmed by the detection of the characteristic optical absorption band in the UV-visible region (plasmon band at ca. 420 nm). TEM analysis of the AgNPs revealed that the particles have spherical shape and polydispersed pattern, with an average size of dimensions of 5–35 nm. Moreover, X-ray diffraction pattern confirmed that NPs are face-centered cubic in shape and FTIR indicated the presence of reducing enzymes and capping proteins on the NPs surface (Fayaz et al. 2011).

Nowadays, several different bacteria have been extensively used to synthesize AgNPs. Table 11.1 summarizes some important and recent studies based on the preparation of AgNPs by different organisms, including bacteria.

11.2.2 Yeasts Synthesis

Compared with other microorganisms, such as bacteria and fungi, there are a few reports describing the use of yeasts to produce AgNPs. In fact, yeasts can be successfully employed to synthesize metallic NPs, and this approach should be more investigated. Extracellular synthesis of AgNPs was observed in silver-tolerant yeast strains MKY3 when challenged with 1 mM silver ions, in the log phase of yeast growth (Kowshik et al. 2003). Recently, biosynthesis of AgNPs by using yeast biomass was reported (Mourato et al. 2011). Extremophilic yeast strain was isolated from acid mine drainage in Portugal. Exposition of washed yeast cells with Ag ions produced AgNPs with diameter size smaller than 20 nm (Mourato et al. 2011).

11.2.3 Fungal Synthesis

Mycosynthesis is the synthesis of nanoparticles by fungi. The term "mycosynthesis" was used for the first time by Ingle et al. (2008) describing the synthesis of nanoparticles by *Fusarium acuminatum*. Overall, fungi possess some important advantages as a potent microorganism for the synthesis of AgNPs. It is relatively easy to culture fungi, the NPs synthesis is mostly extracellular, and the synthesized NPs have a good polydispersivity, size, and stability (Gade et al. 2010a; Birla et al. 2009).

Rai et al. (2009) proposed the term "Myconanotechnology" to include research carried out on nanoparticles synthesized by fungi. Many fungal species have been

Table 11.1 List of important organisms that synthesize silver nanoparticles (AgNPs)

Microorganism	Size (nm)	Reference
Bacteria		
Rhodobacter sphaeroides	9.5 ± 0.3	Bai et al. (2011)
Geobacillus stearothermophilus	5–35	Fayaz et al. (2011)
Brevibacterium casei	10–50	Kalishwaralal et al. (2010)
Pseudomonas aeruginosa	13 ± 3.7	Kumar and Mamidyala (2011)
Morganella spp.	10–50	Parikh et al. (2011)
Bacillus sp.	5–15	Pugazhenthiran et al. (2009)
Escherichia coli	15–50	Zaki et al. (2011)
Bacillus megaterium	15–50	Zaki et al. (2011)
Acinetobacter sp.	15–50	Zaki et al. (2011)
Stenotrophomonas maltophilia	15–50	Zaki et al. (2011)
Continuation-yeasts		
Extremophilic yeast strain	<20	Mourato et al. (2011)
Yeast strain MKY3	2–5	Kowshik et al. (2003)
Fungi		
Rhizopus stolonifer	3–20	Banu et al. (2011)
Phoma glomerata	60–80	Birla et al. (2009)
Geotricum sp.	30–50	Jebali et al. (2011)
Puccinia graminis	30–120	Kirthi et al. (2012)
Aspergillus tamarii	25–50	Kumar et al. (2012a)
Penicillium purpurogenum	8–10	Nayak et al. (2011)
Cochliobolus lunatus	3–21	Salunkhe et al. (2011)
Chrysosporium keratinophilum	24–51	Soni and Prakash (2012)
Verticillium lecanii	20–50	Soni and Prakash (2012)
Fusarium oxysporum f.sp.	20–40	Soni and Prakash (2012)
Phaenerochaete chrysosporium	5–200	Vigneshwaran et al. (2006)
Cladosporium cladosporioides	10–100	Balaji et al. (2009)
Algae		
Chlamydomona reinhardtii	5–35	Barwal et al. (2011)
Gracilaria dura	4–8	Shukla et al. (2012)
Spirulina platensis	6–10	Govindaraju et al. (2008)
Plant extracts		
Rhizophora apiculata	19–42	Antony et al. (2011)
Iresine herbstii	44–64	Dipankar and Murugan (2012)
Tribulus terrestris	16–28	Gopinath et al. (2012)
Boswellia serrata	7.5 ± 3.8	Kora et al. (2012)
Terminalia Chebula	≤100	Kumar et al. (2012b)
Prosopis juliflora	11–19	Raja et al. (2012)
Tridax procumbens	≤20	Rajasekharreddy et al. (2010)
Jatropha curcas	≤20	Rajasekharreddy et al. (2010)
Calotropis gigantea	≤20	Rajasekharreddy et al. (2010)
Solanum melongena	20–40	Rajasekharreddy et al. (2010)
Datura metel	20–40	Rajasekharreddy et al. (2010)
Carica papaya	20–40	Rajasekharreddy et al. (2010)
Citrus aurantium	20–40	Rajasekharreddy et al. (2010)
Ocimum sanctum	6–110	Rao et al. (2013)

(continued)

Table 11.1 (continued)

Microorganism	Size (nm)	Reference
Cocos nucifera coir	23 ± 2	Roopan et al. (2013)
Cissus quadrangularis	50–1,000	Valli and Vaseeharan (2012)
Artemisia nilagirica	70–90	Vijayakumar et al. (2013)
Annona squamosa	20–100	Vivek et al. (2012)
Stevia rebaudiana	2–50	Yilmaz et al. (2011)

explored for the production of different metal nanoparticles of different shapes and sizes. Chen et al. (2003) reported the extracellular formation of AgNPs using *Phoma* species. Some soil-borne fungi like *Aspergillus fumigatus* are able to synthesize AgNPs when the cell extract was challenged with aqueous silver ions (Bhainsa and D'souza 2006). Among different fungal genera used for the synthesis of NPs, the genus *Fusarium* was extensively used. Ahmad et al. (2003) used for the first time the *Fusarium oxysporum* for the synthesis of AgNPs and opened a new avenue in the field of nanobiotechnology. Durán et al. (2005) studied the extracellular production of metal NPs by several strains of the fungus *F. oxysporum*. Similarly, other *Fusarium* species like *F. oxysporum* strain 5115 (Mohammadian et al. 2007), *F. semitectum* (Basavaraja et al. 2008), *F. acuminatum* (Ingle et al. 2008), *F. solani* (Ingle et al. 2009), and *F. culmorum* (Bawaskar et al. 2010) have been successfully used for the synthesis of AgNPs.

Recently, Kumar et al. (2012a) reported the mycogenic synthesis of AgNPs by *Aspergillus tamari*. Ag ions were reduced by the fungal extracellular filtrate leading to the formation of AgNPs, after 30 min of incubation. SEM images revealed that the nanoparticles were spherical with size ranging from 25 to 50 nm. UV-visible spectrophotometry confirmed the presence of the characteristic plasmon band at 420 nm; X-ray diffraction confirmed the crystalline face-centered cubic of AgNPs. Moreover, FTIR indicated that AgNPs were coated with proteins secreted by the fungus, allowing stabilization of AgNPs in aqueous solution (Kumar et al. 2012a). Narayanan and Sakthivel (2010) discussed in detail the biogenic syntheses of metallic nanoparticles, including AgNPs by microbes, in particular by fungi. Table 11.1 summarizes recent important papers based on the mycosynthesis of AgNPs.

11.2.4 Algae Synthesis

The unicellular algae *Chlamydomonas reinhardtii* was used as a model system to investigate the role of cellular proteins in the synthesis of AgNPs (Barwal et al. 2011). Cell-free extract (in vitro) of *C. reinhardtii* and in vivo cells produced AgNPs of size range 5 ± 1 to 15 ± 2 nm and 5 ± 1 to 35 ± 5 nm, respectively. The authors have identified several cellular proteins related to the synthesis of AgNPs by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS), such as ATP

synthase, superoxide dismutase, carbonic anhydrase, and ferredoxin-$NADP^+$ reductase. This elegant paper provides an evidence for the involvement of oxidoreductive proteins in biosynthesis and stabilization of AgNPs (Barwal et al. 2011).

Shukla et al. (2012) reported the formation of AgNPs by the reduction of silver ions, at room temperature, with the addition of agar extracted from the red algae *Gracilaria dura*. AgNPs were found to be spherical in shape, with a good polydispersivity with average size of 6.0 ± 2 nm, as reveled by TEM analysis. Similarly, Govindaraju et al. (2008) reported the formation of several metallic nanoparticles, including AgNPs from *Spirulina platensis*.

11.2.5 Plants Synthesis

Rai et al. (2008) stated that plants have emerged as a simple, cost-effective and eco-friendly system to rapidly synthesize NPs. In this scenario, Persimmon (*Diospyros kaki*) leaf extract led to the formation of AgNPs of 15–90 nm size (Song and Kim 2008). Krishnaraj et al. (2010) reported the rapidly biosynthesis of AgNPs (within 30 min) using leaf extract of *Acalypha indica* and their activity on water-borne bacterial pathogens. Antibacterial activity of biogenically synthesized AgNPs showed effective inhibitory activity against water-borne pathogens, viz., *Escherichia coli* and *Vibrio cholera* (Krishnaraj et al. 2010).

Gade et al. (2010b) reported the green synthesis of AgNPs by *Opuntia ficus-indica*. They evaluate antibacterial activity of synthesized AgNPs against *E. coli* and *S. aureus*, in combination with commercially available antibiotics, such as ampicillin, gentamicin, kanamycin, streptomycin, and vancomycin. Antibacterial activities of the antibiotics increased due to combination with AgNPs, since AgNPs are known to possess antimicrobial properties. The authors also proposed a mechanism for the biogenic synthesis of AgNPs, which involved the biomolecule quercetin (Gade et al. 2010b). Recently, Kumar et al. (2012b) reported the reduction of silver ions to AgNPs by aqueous extract of *Terminalia chebula*, within only 20 min. The formation of AgNPs was confirmed by surface plasmon resonance at 452 nm using UV-visible spectrophotometer. In addition, high-resolution TEM (HR-TEM) revealed the formation of anisotropic nanostructures of pentagons, spherical and triangular-shaped AgNPs, with diameter less than 100 nm. Selected area electron diffraction pattern (SAED) confirmed the crystalline nature of AgNPs. Atomic force microscopy (AFM) images indicated the presence of coating comprised by oxidized polyphenols on the surface of AgNPs. Indeed, in biogenic synthesis of AgNPs by plant extracts, polyphenols act as reducing agent and also as a capping material leading to stabilization of AgNPs in aqueous solution (Kumar et al. 2012b). Narayanan and Sakthivel (2011) published detailed and updated review article based on the biogenic synthesis of metallic nanoparticles, including AgNPs by plant extracts and algae. Table 11.1 summarizes some relevant and recent studies based on green synthesis of AgNPs by plant extracts.

11.3 Toxic Effects of Silver Nanoparticles Administrated In Vitro and In Vivo

In recent years, nanotechnology has been rapidly expanding leading to important impacts on different areas such as health, environment, and economy. In fact, the commercialization of products comprised by nanomaterials is now a reality. Among commercially available nanocompounds, AgNPs are the most used (Ahmed et al. 2008), mainly due to their potent microbicidal activity (Durán et al. 2010). The rapid development of nanotechnology has been leading to an increasing concern related to possible toxic effects of nanomaterials, including human health and environmental impacts (Brayner 2008; Panda et al. 2011). In this scenario, the detailed investigation of nanoparticles toxicity has been emerging as an important area of research. The great interest of the scientific community by toxicological evaluations of nanomaterials is relatively new, and it has been increasing in recent years. However, detailed studies on nanoparticles toxicity, including AgNPs, are still limited and relatively unexplored (de Lima et al. 2012). Therefore, the great appeal of nanotechnology can be considered the better evaluation of the cytotoxicity and genotoxicity of nanoparticles, in particular, biogenic synthesized metallic nanoparticles. In this scenario, this section summarizes a survey of recent evaluations of cytotoxicity and genotoxicity of AgNPs, in special biogenic synthesized AgNPs, and highlights the necessity of more studies in the field.

11.3.1 Culture Cells

Vivek et al. (2012) reported the in vitro cytotoxicity of biogenic AgNPs synthesized by plant extract of *Annona squamosa*. The AgNPs synthesized by plant leaf extract showed to be spherical in shape with average diameter ranging from 20 to 100 nm. The in vitro cytotoxicity of these biogenic AgNPs were evaluated against normal epithelial cells (HBL-100) and human breast cancer cell (MCF-7). The authors reported a dose-dependent cytotoxicity effect. The inhibitory concentrations (IC50), in which 50 % of cells die, were found to be 50 μg/mL, 30 μg/mL and 80 μg/mL, 60 μg/mL for AgNPs against MCF-7 and normal HBL-100 cells at 24- and 48-h incubation, respectively (Vivek et al. 2012). Moreover, it was also observed an induction of apoptosis. Overall, the results revealed cytotoxic effects of biosynthesized AgNPs against breast cancer cell line, in comparison with normal breast cell line, indicating that these nanoparticles might be used in cancer treatments.

A similar work describes the preparation of biogenic AgNPs by plant extract of *Iresine herbstii* (Dipankar and Murugan 2012). The obtained AgNPs were found to be face-centered cubic in shape, with size ranging from 44 to 64 nm, and capped with plant components (Dipankar and Murugan 2012). The in vitro cytotoxicity of plant-mediated AgNPs was evaluated towards HeLa cancer cells with the trypan blue assay. AgNPs exhibited a potent toxic effect (CL_{50} of 51 μg/mL) on cancer

cells with 88 % death of HeLa cells upon treatment with 300 μg/mL of AgNPs (Dipankar and Murugan 2012).

However, the toxicity of AgNPs towards culture cells is still under study. Several studies did not observe toxicity on human culture cells treated with capped AgNPs (diameters ranging from 6 to 80 nm) below 10 mg/mL (Hussain et al. 2005; AshaRani et al. 2009; Lu et al. 2010; Foldbjerg et al. 2011). An interesting paper investigated the toxicity of biogenic AgNPs (size of 25–45 nm) synthesized by *Alternaria alternata* towards human lymphocytes using comet assay (Sarkar et al. 2011). The authors reported that up to 50 mg/mL of biogenic AgNPs, no DNA damage was observed. DNA damage was only reported upon incubation of human lymphocytes with over 300 mg/mL of AgNPs.

On contrary, some papers described different degrees of toxicity upon incubation of cell cultures with AgNPs, at concentrations up to 50 mg/mL (de Lima et al. 2012). For example, culture of human mesenchymal stem cells incubated with 0.1 mg/mL of albumin-capped AgNPs (average size 46 nm) (Hackenberg et al. 2011) or human glioblastoma cells starch incubated with capped AgNPs (sizes 6–20 nm) (AshaRani et al. 2008) showed genotoxicity up to 50 mg/mL of AgNPs. Another interesting work showed that albumin-coated AgNPs (average size 70 nm) were found to be more genotoxic on mouse peritoneal macrophage cell line (genotoxicity at around 2 mg/mL) (Park et al. 2010) in comparison with AgNPs capped with polysaccharides (average size 25 nm), on mouse embryonic stem and fibroblasts cells, which exhibited genotoxicity at 50 mg/mL (Ahmed et al. 2008). In general and with a few exceptions, human culture cells were observed to be less sensitive compared to mouse culture cells, upon treatment with capped AgNPs, independently of the chemical nature of the capping (de Lima et al. 2012). Obviously, this tendency should be further investigated.

11.3.2 Calf Thymus DNA

It was observed that the genotoxicity of AgNPs alone (sizes 20–50 nm) was weak on calf thymus DNA (ctDNA); however, in the presence of detergent (cetylpyridinium bromide), the NPs showed significant genotoxicity, mainly owing to the presence of the surfactant (Chi et al. 2009). This result is important from an environmental point of view, since considerable amounts of AgNPs are eliminated to water (rivers, oceans) and probably readily interacting with surfactant presented in the water, leading to a strong genotoxicity owing to the formation of AgNPs–detergent interactions (Chi et al. 2009).

11.3.3 In Vivo Assays

Many papers have reported in vivo toxicity studies of AgNPs by different routes of administration (Tian et al. 2007; Vlachou et al. 2007; Trop et al. 2006; Samberg et al. 2010). For example, Rahman et al. (2009) reported that intraperitoneal

administration of AgNPs may lead to alterations of gene expression, suggesting the neurotoxic effects of these nanoparticles. Furthermore, several reports describe oral administration of AgNPs (Cha et al. 2008; Kim et al. 2009a, b). After oral exposure, AgNPs and/or silver ions are assumed to translocate from the gut into the blood, systemically inducing liver damage. However, it is necessary to carefully investigate these statements, since it is necessary to distinguish whether the systemic distribution of silver is due to the presence of AgNPs or silver ions in the liver, and this topic deserves more studies, as stated by Johnston et al. (2010). Concerning to the possible oxidation of AgNPs to silver ions, an in vivo study based on intravenous administration of AgNPs on mouse supported the antiplatelet properties of these nanoparticles (AgNPs are considered to have antiplatelet properties) (Shrivastava et al. 2009). It was suggested that there is a low possibility of oxidation of AgNPs into silver ions inside the platelets due to the high silver ionization potential (735 kJ mol^{-1}), since this high ionization potential is difficult to reach in an intracellular environment. In view of this fact the observed antiplatelet property effects are more likely to be due to AgNPs rather than to silver ions. It is noticed that this same effect was observed in human platelets (patients with type 2 diabetes mellitus) (Shrivastava et al. 2009).

In a recent study, the mycosynthesis of AgNPs by filamentous fungus *Cochliobolus lunatus*, their characterization, and larvicidal effects were reported (Salunkhe et al. 2011). Toxic effects of biogenic AgNPs towards *Aedes aegypti* and *Anopheles stephensi*, which are responsible for diseases of public health importance, were investigated. Potent mortality effects on second, third, and fourth instar larvae of *A. aegypti* and *A. stephensi* were found to correlate with concentrations of AgNPs. The observed larvicidal activity of mycogenic AgNPs was related to the penetration of nanoparticles through larvae membrane. Moreover, toxicity studies were also carried out against nontarget fish species *Poecilia reticulata*. Common organisms found in this kind of habits are *A. aegypti* and *A. stephensi*. Interestingly, mycogenic AgNPs did not exhibit noticeable effects on *P. reticulata* after either 24 or 48 h of exposure at the level of LC50 and LC90 values against fourth instar larvae of *A. aegypti* and *A. stephensi*. These results indicate that biogenic AgNPs might be used as a potent larvicidal agent, with no toxic effects to the environment (Salunkhe et al. 2011).

Interestingly, recent studies suggested that in vivo toxicity of AgNPs is dependent on the organism. In this scenario, in vivo studies demonstrated that mice are more sensitive to capped AgNPs in comparison with fish. Indeed, some papers showed that AgNPs capped with starch (8–15 nm), or with bovine serum albumin (10–20 nm) (AshaRani et al. 2008), or with polyvinyl alcohol (5–35 nm) (AshaRani et al. 2011) were no genotoxic towards zebra fish embryos up to nanoparticles concentration of 25 mg/mL. Genotoxicity was observed by increasing the Nanoparticles concentration to values over 100 mg/mL, in special in the case of albumin-capped AgNPs, which lead to embryos apoptosis and also abnormalities (AshaRani et al. 2008). However, at low nanoparticle dose, such as 1 mg mL^{-1}, this effect was found to be very discrete (Ordzhonikidze et al. 2009).

Very few studies investigated the pulmonary toxicity of AgNPs. As stated by Johnston et al. (2010), there is limited information available related to this topic. Studies based on the investigation of pulmonary toxicity of AgNPs are important since proinflammatory and oxidative potentials of AgNPs within the lung are known to drive nanoparticles toxicity (Sung et al. 2009; Hyun et al. 2008; Ji et al. 2007). Aspects such as exposure time to nanoparticles, their concentrations, routes of applications, and particle sizes are extremely important to define nanoparticle toxicity. AgNP translocation and their potential accumulation within secondary targets, such as liver, spleen, and brain, following pulmonary exposure, were reported. However, Johnston et al. (2010) stated that, until now, it is not possible to confirm that the silver content of any cell distribution is due to AgNPs or to silver ions, since the methods used are not sensible enough to discriminate between them. It is known that in biological systems both forms of silver can exist owing to degradation and/or metabolism of the nanoparticles, indicating that further studies are required in this field.

Moreover, several applications of AgNPs are based on dermatological uses, such as the production of wound dressing containing AgNPs. In this regards, it is important to further investigate possible toxic effects associated with administration of AgNPs on either wounded skin or normal skin. It is important to clearly define the hazards associated with dermal exposure to AgNPs, since liver has been suggested as a secondary target for nanoparticles toxicity (de Lima et al. 2012 and reference therein).

11.4 Aspects That Determine Nanoparticles Cytotoxicity and Genotoxicity

Several aspects including size, surface area, surface reactivity, composition, surface charge, and capping molecules are known to influence cell toxicity. In general, reactivity of nanomaterials in biological medium is directly related to the observed toxic effects of nanoparticles, and particle size controls the reactivity of these nanomaterials (Brown et al. 2001; Duffin et al. 2007). Indeed, small nanoparticles can cause several side effects in lung, due to their higher ability for cell invasion (Oberdörster et al. 2005). Hence, smaller sized particles were found to be more toxic than bigger sized particles (Panda et al. 2011; Gaiser et al. 2012). However, some exception was reported, as in the case of nonmetallic nanoparticles, such as polymeric nanoparticles. Toxicity of chitosan nanoparticles indicated that smaller particles might be used more safely than larger particles, at higher concentrations, and probably others factors could be involved in nanoparticle toxicity (Lima et al. 2010). Overall, in the case of metallic nanoparticles such as AgNPs, it can be observed a tendency that small particles have higher reactivity and thus produce higher toxicity (Ordzhonikidze et al. 2009; Kim et al. 2011). However, it must be noted that particle size is not the only factor that determines nanoparticle toxicity,

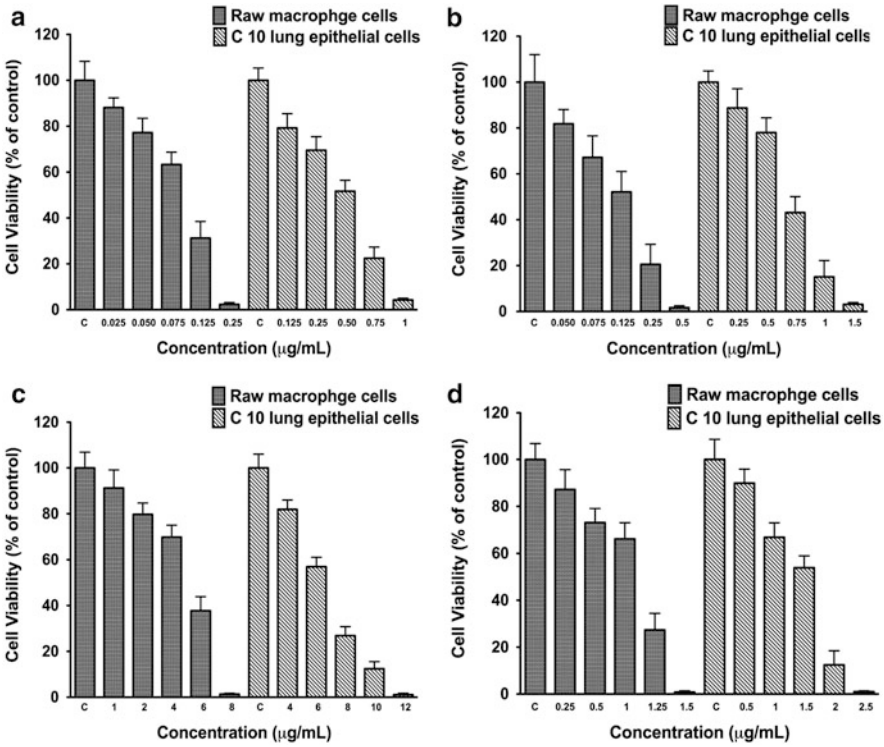

Fig. 11.1 Results of MTT assay of the mouse macrophage and lung epithelial cells exposed to the four different types of silver nanoparticles at various concentrations: (**a**) poly(diallyldimethylammonium) chloride-AgNPs, (**b**) biogenic AgNPs, (**c**) uncoated-AgNps, and (**d**) oleate-Ag nanoparticles NPs. Reproduced from Suresh et al. 2012 by permission of American Chemical Society

since important parameters such as synthesis procedures, presence and nature of capping agents, surface charge, and aggregation are also important (Panda et al. 2011; Suresh et al. 2012). In fact, Suresh et al. (2012) investigated the cytotoxicity of effects of AgNPs with different surface coatings on two different cell lines. Figure 11.1 shows the cytotoxicity of mouse macrophage and lung epithelial cells treated with four different types of AgNPs: (a) poly (diallyldimethylammonium) chloride-AgNPs; (b) biogenic AgNPs, synthesized by bacteria *Shewanella oneidensis*; (c) uncoated-AgNPs (colloidal-AgNPs); and (d) oleate-AgNPs. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay measured mitochondrial integrity and oxidative metabolism and is an indirect measure of cell viability. All different AgNPs tested showed a dose-dependent cytotoxicity in both cell lines. However, depending on the type of AgNPs different cytotoxic parameters were obtained. For example, in the case of mouse macrophage cells, the inhibitory concentration values were found to be 0.1, 0.125, 1.1, and 4.9 μg/mL for poly(diallyldimethylammonium) chloride-AgNPs,

biogenic AgNPs, oleate-AgNPs, and uncoated-AgNPs, respectively. For lung epithelial cells, the inhibitory concentration values were found to be 0.45, 0.7, 1.6, and 6.3 μg/mL for poly(diallyldimethylammonium) chloride-AgNPs, biogenic AgNPs, oleate-AgNPs, and uncoated-AgNPs, respectively. It can be concluded that uncoated-AgNPs were the less toxic to both cell lines, followed by olate-AgNPs, biogenic AgNPs, and poly(diallyldimethylammonium) chloride-AgNPs (Suresh et al. 2012). These results reveal the impact of synthesis procedure and surface coating in determining nanoparticle toxicity.

11.5 Comments and Future Perspectives

It can be concluded that biogenic synthesis of metallic NPs, in particular AgNPs, has been emerging as an important and fruitful domain of nanotechnology. Despite the fact that several papers have successfully described different routes to biogenically synthesize AgNPs, as stated in this chapter, the mechanisms involved in the biogenic production of AgNPs have not yet been elucidated. Therefore, elucidation of the mechanistic aspects of some biological systems in the synthesis of nanoparticles needs more detailed studies (Durán et al. 2011).

Moreover, by reviewing the actual literature based on AgNPs and toxicity, it can be concluded that there are few studies based on cytotoxicity and genotoxicity of biogenic AgNPs, in comparison with chemically synthesized AgNPs. The lack of more studies based on toxicity of biogenic AgNPs can be understood by considering that biogenic synthesis of metallic nanoparticles is a relatively new methodology (de Lima et al. 2012). By analyzing and comparing published papers on toxic effects of AgNPs, the determination of AgNPs toxicity (both cytotoxicity and genotoxicity) is a complex issue, since many different parameters play important roles. In fact, controversial papers showing different effects of toxicity of AgNPs can be understood by considering several parameters, such as different methodologies to synthesize the nanoparticles, nanoparticles size, shape, presence and nature of capping agents, surface area and charge, and finally, the diverse kinds of toxic evaluation tests employed (de Lima et al. 2012). The uses of different organisms and/or culture cells to investigate AgNPs toxicity make the comparison between different studies a difficult and complex task, leading to inconclusive results in some cases. In this context, it is necessary to elaborate standard protocols to carefully analyze nanoparticles toxicity in order to decrease the possible discrepancies related to final conclusions and comparison between different works.

Therefore, with the present information, it is premature to conclude whether biogenic nanoparticles are less genotoxic/cytotoxic compared with chemically synthesized nanoparticles. However, some considerations and tendencies can be postulated with the actual literature, such as the lower toxicity of AgNPs compared with silver ions (Ordzhonikidze et al. 2009; Kim et al. 2011; Griffitt et al. 2008; Park and Choi 2010; Panda et al. 2011; Gaiser et al. 2012). Panda et al. (2011) investigated the induction of cellular death of cultured cells of *Allium cepa*

incubated with silver ions (Ag^+), silver complexes (AgCl), capped biogenic AgNPs (AgNP-biogenic), and commercially uncapped AgNPs from Sigma (AgNP-Sigma), and they found that the induction of cell death followed the order: Ag^+ ions > colloidal AgCl > AgNP-commercial from Sigma > AgNP-biogenic, showing that the less toxic form of silver was biogenic synthesized capped AgNPs. Similar results were reported for biogenic AgNPs for different applications, such as wound healing antileishmaniases, antifungal, and antibacterial effects (Melo et al. 2011; Marcato and Durán 2011; Rossi-Bergmann et al. 2012; Marcato et al. 2012a, b).

Taken together, it can be assumed that biogenic synthesis of AgNPs has been emerging as an attractive and important route to prepare nanoparticles for several applications. In general, biogenic AgNPs are less toxic in comparisons with chemically synthesized nanoparticles. This chapter highlights the necessity of more mechanist studies on the biogenic synthesis of AgNPs, in order to optimize the green synthesis process, and more studies based on the toxicity of AgNPs, in particular, genotoxicity studies of biogenic AgNPs.

Acknowledgement Support from FAPESP, CNPq and Brazilian Network on Nanotoxicology (MCTI/CNPq), and NanoBioss (MCTI) is acknowledged.

References

Ahmad A, Mukherjee P, Senapati S et al (2003) Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids Surf B Biointerfaces 28:313–318

Ahmed M, Karns M, Goodson M et al (2008) DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. Toxicol Appl Pharmacol 233:404–410

Antony JJ, Sivalingam P, Siva D et al (2011) Comparative evaluation of antibacterial activity of silver nanoparticles synthesized using *Rhizophora apiculata* and glucose. Colloids Surf B Biointerfaces 88:134–140

Arora S, Jain J, Rajwade JM et al (2008) Cellular responses induced by silver nanoparticles: *in vitro* studies. Toxicol Lett 79:93–100

AshaRani PV, Wu YL, Gong Z et al (2008) Toxicity of silver nanoparticles in zebrafish models. Nanotechnology 19:255102

AshaRani PV, Mun GLK, Hande MP et al (2009) Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano 3:279–290

AshaRani PV, Lianwu Y, Gong Z et al (2011) Comparison of the toxicity of silver, gold and platinum nanoparticles in developing zebrafish embryos. Nanotoxicology 5:43–54

Bai H-J, Yang B-S, Chai C-J et al (2011) Green synthesis of silver nanoparticles using *Rhodobacter sphaeroides*. World J Microbiol Biotechnol 27:2723–2728

Balaji DS, Basavaraja S, Deshpande R et al (2009) Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium cladosporioides* fungus. Colloids Surf B Biointerfaces 68:88–92

Bansal V, Bharde A, Ramanathan R et al (2012) Inorganic materials using 'unusual' microorganisms. Adv Colloid Interface Sci 179:150–168

Banu A, Rathod V, Ranganath E (2011) Silver nanoparticle production by *Rhizopus stolonifer* and its antibacterial activity against extended spectrum b-lactamase producing (ESBL) strains of Enterobacteriaceae. Mater Res Bull 46:1417–1423

Barwal I, Ranjan P, Kateriya S et al (2011) Cellular oxido-reductive proteins of *Chlamydomonas reinhardtii* control the biosynthesis of silver nanoparticles. J Nanobiotechnology 9:56. doi:10.1186/1477-3155-9-56

Basavaraja S, Balaji SD, Legashetty A et al (2008) Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum*. Mater Res Bull 43:1164–1170

Bawaskar M, Gaikwad S, Ingle A et al (2010) A new report on mycosynthesis of silver nanoparticles by *Fusarium culmorum*. Curr Nanosci 6:376–380

Bhainsa KC, D'souza SK (2006) Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. Colloids Surf B Biointerfaces 47:160–164

Birla SS, Tiwari VV, Gade AK et al (2009) Fabrication of silver nanoparticles by *Phoma glomerata* and its combined effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Lett Appl Microbiol 48:173–179

Brayner R (2008) The toxicological impact of nanoparticles. Nano Today 3:48–55

Brown DM, Wilson MR, MacNee W et al (2001) Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. Toxicol Appl Pharmacol 175:191–199

Cha K, Hong HW, Choi YG et al (2008) Comparison of acute responses of mice livers to short-term exposure to nano-sized or micro-sized silver particles. Biotechnol Lett 30:1893–1899

Chen JC, Lin ZH, Ma XX (2003) Evidence of the production of silver nanoparticles via pretreatment of *Phoma* sp.3.2883 with silver nitrate. Lett Appl Microbiol 37:105–108

Chi Z, Li R, Zhao L et al (2009) A new strategy to probe the genotoxicity of silver nanoparticles combined with cetylpyridine bromide. Spectrochim Acta A Mol Biomol Spectrosc 72:577–581

de Lima R, Seabra AB, Durán N (2012) Silver nanoparticles: a brief review of cytotoxicity and genotoxicity of chemically and biogenically synthesized nanoparticles. J Appl Toxicol 32:867–879

Dipankar C, Murugan S (2012) The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from *Iresine herbstii* leaf aqueous extracts. Colloids Surf B Biointerfaces 98:112–119

Doak SH, Manshian B, Jemkins GJ et al (2012) In vitro genotoxicity testing strategy for nanomaterials and the adaptation of current OECD guidelines. Mutat Res 745:104–111

Donaldson K, Stone V, MacNee W (1999) The toxicology of ultrafine particles. In: Maynard LA, Howards CA (eds) Particulate matter properties and effects upon health. Bios Scientific, Oxford, pp 115–127

Duffin R, Tran L, Brown D et al (2007) Proinflammogenic effects of low-toxicity and metal nanoparticles *in vivo* and *in vitro*: highlighting the role of particle surface area and surface reactivity. Inhal Toxicol 19:849–856

Durán N, Marcato PD, Alves OL et al (2005) Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. J Nanobiotechnol 3:8–14

Durán N, Marcato PD, de Conti R et al (2010) Potential use of silver nanoparticles on pathogenic bacteria, their toxicity and possible mechanism of action. J Braz Chem Soc 21:949–959

Durán N, Marcato PD, Durán M et al (2011) Mechanistic aspects in the biogenic synthesis of extracellular metal nanoparticles by peptides, bacteria, fungi, and plants. Appl Microbiol Biotechnol 90:1609–1624

Fayaz AM, Girilal M, Rahman M et al (2011) Biosynthesis of silver and gold nanoparticles using thermophilic bacterium *Geobacillus stearothermophilus*. Process Biochem 46:1958–1962

Foldbjerg R, Dang DA, Autrup H (2011) Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. Arch Toxicol 85:743–750

Gade A, Ingle A, Whiteley C et al (2010a) Mycogenic metal nanoparticles: progress and applications. Biotechnol Lett 32:593–600

Gade A, Gaikwad S, Tiwari V et al (2010b) Biofabrication of silver nanoparticles by *Opuntia ficus-indica*: *in vitro* antibacterial activity and study of the mechanism involved in the synthesis. Curr Nanosci 6:370–375

Gaiser BK, Fernandes TF, Jepson MA et al (2012) Interspecies comparisons on the uptake and toxicity of silver and cerium dioxide nanoparticles. Environ Toxicol Chem 31:144–154
Gonzalez L, Sanderson BJ, Kirsh-Volders M (2011) Adaptations of the in vitro MN assay for the genotoxicity assessment of nanomaterials. Mutagenesis 26:185–191
Gopinath V, MubarakAli D, Priyadarshini NM et al (2012) Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: a novel biological approach. Colloids Surf B Biointerfaces 96:69–74
Govindaraju K, Basha SK, Kumar G et al (2008) Silver, gold and bimetallic nanoparticles production using single-cell protein (*Spirulina platensis*) Geitler. J Mater Sci 43:5115–5122
Griffitt RJ, Luo J, Gao J et al (2008) Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. Environ Toxicol Chem 27:1972–1978
Gurr JR, Wang AS, Chen CH et al (2005) Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology 213:66–73
Hackenberg S, Scherzed A, Kessler M et al (2011) Silver nanoparticles: evaluation of DNA damage, toxicity and functional impairment in human mesenchymal stem cells. Toxicol Lett 201:27–33
Hood E (2004) Nanotechnology, diving into the unknown. Environ Health Perspect 112: A747–A749
Hussain SM, Hess KL, Gearhart JM et al (2005) *In vitro* toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol In Vitro 19:975–983
Hyun JS, Lee BS, Ryu HY et al (2008) Effects of repeated silver nanoparticles exposure on the histological structure and mucins of nasal respiratory mucosa in rats. Toxicol Lett 182:24–28
Ingle A, Gade A, Pierrat S et al (2008) Mycosynthesis of silver nanoparticles using the fungus *Fusarium acuminatum* and its activity against some human pathogenic bacteria. Curr Nanosci 4:141–144
Ingle A, Gade A, Bawaskar M et al (2009) *Fusarium solani*: a novel biological agent for the extracellular synthesis of silver nanoparticles. J Nanopart Res 11:2079–2085
Jebali A, Ramezani F, Kazemi B (2011) Biosynthesis of silver nanoparticles by *Geotrichum* sp. J Cluster Sci 22:225–232
Ji JH, Jung JH, Kim SS et al (2007) Twenty-eight-day inhalation toxicity study of silver nanoparticles in Sprague–Dawley rats. Inhal Toxicol 19:857–871
Joerger R, Klaus T, Granqvist CG (2000) Biologically produced silver-carbon composite materials for optically functional thin film coatings. Adv Mater 12:l407–l409
Johnston HJ, Hutchison G, Christensen FM et al (2010) A review of the *in vivo* and *in vitro* toxicity of silver and gold particulates: particle attributes and biological mechanisms responsible for the observed toxicity. Crit Rev Toxicol 40:328–346
Ju-Nam Y, Lead JR (2008) Manufactured nanoparticles: an overview of their chemistry, interactions and potential environmental implications. Sci Total Environ 400:396–414
Kalishwaralal K, Deepaka V, Pandiana SR et al (2010) Biosynthesis of silver and gold nanoparticles using *Brevibacterium casei*. Colloids Surf B Biointerfaces 77:257–262
Kim S, Choi JE, Cho J et al (2009a) Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. Toxicol In Vitro 23:1076–1084
Kim Y, Suh HS, Cha HJ et al (2009b) A case of generalized argyria after ingestion of colloidal silver solution. Am J Ind Med 52:246–250
Kim JS, Sung JH, Ji JH et al (2011) *In vivo* genotoxicity of silver nanoparticles after 90-day silver nanoparticle inhalation exposure. Saf Health Work 2:34–38
Kirthi AV, Rahuman AA, Jayaseelan C et al (2012) Novel approach to synthesis silver nanoparticles using plant pathogenic fungi, *Puccinia graminis*. Mater Lett 81:69–72
Klaus T, Granqvist CG, Joerger R et al (1999) Silver-based crystalline nanoparticles, microbially fabricated. Proc Natl Acad Sci U S A 96:13611–13614

Klaus-Joerger T, Joerge R, Olsson E et al (2001) Bacteria as workers in the living factory: metal accumulating bacteria and their potential for material sciences a review. Trends Biotechnol 19:15–20

Kora AJ, Sashidhar RB, Arunachalama J (2012) Aqueous extract of gum olibanum (*Boswellia serrata*): a reductant and stabilizer for the biosynthesis of antibacterial silver nanoparticles. Process Biochem 47:1516–1520

Kowshik M, Ashtaputre S, Kharrazi S et al (2003) Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3. Nanotechnology 14:95–100

Krishnaraj C, Jagan EG, Rajasekar S et al (2010) Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B Biointerfaces 76:50–56

Kumar CG, Mamidyala SK (2011) Extracellular synthesis of silver nanoparticles using culture supernatant of *Pseudomonas aeruginosa*. Colloids Surf B Biointerfaces 84:462–466

Kumar RR, Priyadharsani KP, Thamaraiselvi K (2012a) Mycogenic synthesis of silver nanoparticles by the Japanese environmental isolate *Aspergillus tamari*. J Nanopart Res 14:860. doi:10.1007/s11051-012-0860-2

Kumar KM, Sinha M, Mandal BK et al (2012b) Green synthesis of silver nanoparticles using *Terminalia chebula* extract at room temperature and their antimicrobial studies. Spectrochim Acta A Mol Biomol Spectrosc 91:228–233

Lewinski N, Colvin V, Drezedk R (2008) Cytotoxicity of nanoparticles. Small 4:26–49

Li X, Xu H, Chen Z-S et al (2011) Biosynthesis of nanoparticles by microorganisms and their applications. J Nanomater Article ID 270974. doi:10.1155/2011/270974

Lima R, Feitosa L, Pereira AES et al (2010) Evaluation of the genotoxicity of chitosan nanoparticles for use in food packaging films. J Food Sci 75:89–96

Lu W, Senapati D, Wang S et al (2010) Effect of surface coating on the toxicity of silver nanomaterials on human skin keratinocytes. Chem Phys Lett 487:92–96

Marcato PD, Durán N (2011) Biogenic silver nanoparticles: applications in medicines and textiles and their health implications. In: Rai M, Durán N (eds) Metal nanoparticles in microbiology, Springer, Germany, Chap. 11, p 249–267

Marcato PD, Durán M, Huber SC et al (2012a) Biogenic silver nanoparticles and its antifungal activity as a new topical transungual drug. J Nano Res 20:99–107

Marcato PD, Nakasato G, Brocchi M et al (2012b) Biogenic silver nanoparticles: antibacterial and cytotoxicity applied to textile fabrics. J Nano Res 20:69–76

Melo PS, Marcato PD, Huber SC, Ferreira IR, de Paula LB, Almeida ABA, Durán N, Torloni S, Seabra AB, Alves OL (2011) Nanoparticles in treatment of thermal injured rats: is it safe? J Phys Conf Ser 304:012027

Mohammadian A, Shojaosadati SA, Habibi-Rezaee M (2007) *Fusarium oxysporum* mediates photogeneration of silver nanoparticles. Scientia Iranica 14:323–326

Mourato A, Gadanho M, Lino AR et al (2011) Biosynthesis of crystalline silver and gold nanoparticles by extremophilic yeasts. Bioinorg Chem Appl Article ID 546074. doi:10.1155/2011/546074

Narayanan KB, Sakthivel N (2010) Biological synthesis of metal nanoparticles by microbes. Adv Colloid Interface Sci 156:1–13

Narayanan KB, Sakthivel N (2011) Green synthesis of biogenic metal nanoparticles by terrestrial and aquatic phototrophic and heterotrophic eukaryotes and biocompatible agents. Adv Colloid Interface Sci 169:59–79

Nayak RR, Pradhan N, Behera D et al (2011) Green synthesis of silver nanoparticle by *Penicillium purpurogenum* NPMF: the process and optimization. J Nanopart Res 13:3129–3137

Nel A, Xia T, Madler L et al (2006) Toxic potential of materials at the nanolevel. Science 311:622–627

Ng CT, Li JJ, Bay BH et al (2010) Current studies into the genotoxic effects of nanomaterials. J Nucleic Acid Article ID 947859. doi: 10.4061/2010/947859

Oberdörster E (2004) Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile largemouth bass. Environ Health Perspect 112:1058–1062
Oberdörster G, Oberdörster E, Oberdörster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113:823–839
Ordzhonikidze CG, Ramaiyya LK, Egorova EM et al (2009) Genotoxic effects of silver nanoparticles on mice *in vivo*. Acta Naturae (Russia) 3:99–101
Panda KK, Achary VMM, Krishnaveni R et al (2011) *In vitro* biosynthesis and genotoxicity bioassay of silver nanoparticles using plants. Toxicol In Vitro 25:1097–1105
Parikh RY, Ramanathan R, Coloe PJ et al (2011) Genus-wide physicochemical evidence of extracellular crystalline silver nanoparticles biosynthesis by *Morganella* spp. PLoS ONE 6: e21401
Park BSY, Choi J (2010) Geno- and ecotoxicity evaluation of silver nanoparticles in freshwater crustacean Daphnia magna. Environ Eng Res 15:23–27
Park AEJ, Yi J, Kim Y et al (2010) Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism. Toxicol In Vitro 24:872–878
Prakasham RS, Kumar BS, Kumar YS et al (2012) Characterization of silver nanoparticles synthesized by using marine isolate *Streptomyces albidoflavus*. J Microbiol Biotechnol 22:614–621
Pugazhenthiran N, Anandan S, Kathiravan G et al (2009) Microbial synthesis of silver nanoparticles by *Bacillus* sp. J Nanopart Res 11:1811–1815
Rahman MF, Wang J, Patterson TA et al (2009) Expression of genes related to oxidative stress in the mouse brain after exposure to silver-25 nanoparticles. Toxicol Lett 187:15–21
Rai M, Yadav A, Gade A (2008) CRC 675—currents trends in phytosynthesis of metal nanoparticles. Crit Rev Biotechnol 28:277–284
Rai M, Yadav A, Bridge P et al (2009) Myconanotechnology: a new and emerging science. Appl Mycol 258–267
Raja K, Saravanakumar A, Vijayakumar R (2012) Efficient synthesis of silver nanoparticles from *Prosopis juliflora* leaf extract and its antimicrobial activity using sewage. Spectrochim Acta A Mol Biomol Spectrosc 97:490–494
Rajasekharreddy P, Rani PU, Sreedhar B (2010) Qualitative assessment of silver and gold nanoparticle synthesis in various plants: a photobiological approach. J Nanopart Res 12:1711–1721
Rao YS, Kotakadi VS, Prasad TNVKV et al (2013) Green synthesis and spectral characterization of silver nanoparticles from Lakshmi tulasi (*Ocimum sanctum*) leaf extract. Spectrochim Acta A Mol Biomol Spectrosc 103:156–159
Rico CM, Majumdar S, Duarte-Gardea M et al (2011) Interaction of nanoparticles with edible plants and their possible implications in the food chain. J Agric Food Chem 59:3485–3498
Roopan SM, Madhumitha RG, Rahuman AA et al (2013) Low-cost and eco-friendly phyto-synthesis of silver nanoparticles using *Cocos nucifera* coir extract and its larvicidal activity. Ind Crop Prod 43:631–635
Rossi-Bergmann B, Pacienza-Lima W, Marcato PD et al (2012) *In vivo* Leishmaniasis Study: biogenic and chemical silver nanoparticles. J Nano Res 20:89–97
Salunkhe RB, Patil SV, Patil CD et al (2011) Larvicidal potential of silver nanoparticles synthesized using fungus *Cochliobolus lunatus* against *Aedes aegypti* (Linnaeus, 1762) and *Anopheles stephensi* Liston (Diptera; Culicidae). Parasitol Res 109:823–831
Samberg ME, Oldenburg SJ, Monteiro-Riviere NA (2010) Evaluation of silver nanoparticle toxicity in skin *in vivo* and keratinocytes *in vitro*. Environ Health Perspect 118:407–413
Sarkar J, Chattopadhyay D, Patra S et al (2011) *Alternaria alternata* mediated synthesis of protein capped silver nanoparticles and their genotoxic activity. Dig J Nanomater Biostruct 6:563–573
Shrivastava S, Bera T, Singh SK et al (2009) Characterization of antiplatelet properties of silver nanoparticles. ACS Nano 3:1357–1364

Shukla MK, Singh RP, Reddy CRK et al (2012) Synthesis and characterization of agar-based silver nanoparticles and nanocomposite film with antibacterial applications. Bioresour Technol 107:295–300

Song JY, Kim BS (2008) Biological synthesis of bimetallic Au/Ag nanoparticles using Persimmon (*Diopyros kaki*) leaf extract. Korean J Chem Eng 25:808–811

Soni N, Prakash S (2012) Fungal-mediated nano silver: an effective adulticide against mosquito. Parasitol Res 111:2091–2098

Sung JH, Ji JH, Park JD et al (2009) Subchronic inhalation toxicity of silver nanoparticles. Toxicol Sci 108:452–461

Suresh AK, Pelletier DA, Wang W et al (2012) Cytotoxicity induced by engineered silver nanocrystallites is dependent on surface coatings and cell types. Langmuir 28:2727–2735

Tian J, Wong KK, Ho CM et al (2007) Topical delivery of silver nanoparticles promotes wound healing. ChemMedChem 2:129–136

Trop M, Novak M, Rodl S et al (2006) Silver coated dressing acticoat caused raised liver enzymes and argyria-like symptoms in burn patient. J Trauma 60:648–665

Valli JS, Vaseeharan B (2012) Biosynthesis of silver nanoparticles by *Cissus quadrangularis* extracts. Mater Lett 82:71–173

Vigneshwaran N, Kathe AA, Varadarajan PV et al (2006) Biomimetics of silver nanoparticles by white rot fungus, *Phaenerochaete chrysosporium*. Colloids Surf B Biointerfaces 53:55–59

Vijayakumar M, Priya K, Nancy FT et al (2013) Biosynthesis, characterisation and anti-bacterial effect of plant-mediated silver nanoparticles using *Artemisia nilagirica*. Ind Crop Prod 41:235–240

Vivek R, Thangam R, Muthuchelian K et al (2012) Green biosynthesis of silver nanoparticles from *Annona squamosa* leaf extract and its *in vitro* cytotoxic effect on MCF-7 cells. Process Biochem 47:2405–2410

Vlachou E, Chipp E, Shale E et al (2007) The safety of nanocrystalline silver dressings on burns: a study of systemic silver absorption. Burns 33:979–985

Wijnhoven SWP, Peijnenburg WJGM, Herberts CA et al (2009) Nano-silver—a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology 3:109–138

Wise JP Sr, Goodale BC, Wise SS et al (2010) Silver nanospheres are cytotoxic and genotoxic to fish cells. Aquat Toxicol 97:34–41

Xiong D, Fang T, Yu L et al (2011) Effects of nano-scale TiO_2, ZnO and their bulk counterparts on zebraûsh: acute toxicity, oxidative stress and oxidative damage. Sci Total Environ 409:1444–1452

Yilmaz M, Turkdemir H, Kilic AM et al (2011) Biosynthesis of silver nanoparticles using leaves of *Stevia rebaudiana*. Mater Chem Phys 130:1195–1202

Zaki S, Kady MFE, Abd-El-Haleem D (2011) Biosynthesis and structural characterization of silver nanoparticles from bacterial isolates. Mater Res Bull 46:1571–1576