15 Toxic and Beneficial Potential of Silver Nanoparticles: The Two Sides of the Same Coin

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Abstract

Nanotechnology has allowed great changes in chemical, biological and physical properties of metals when compared to their bulk counterparts. Within this context, silver nanoparticles (AgNPs) play a major role due to their unique properties, being widely used in daily products such as fabrics, washing machines, water filters, food and medicine. However, AgNPs can enter cells inducing a "Trojan-horse" type mechanism which potentially leads to cellular autophagy, apoptosis or necrosis. On the other hand, this cytotoxicity mechanism can be optimized to develop drug nanocarriers and anticancer therapies. The increasing use of these NPs entails their release into the environment, damaging ecosystems balance and representing a threat to human health. In this context, the possible deleterious effects that these NPs may represent for the biotic and abiotic ecosystems components represent an obstacle that must be overcome in order to guarantee the safety use of their unique properties.

Keywords

Silver nanoparticles · AgNPs · Nanotoxicity · Aquatic toxicity · Cytotoxicity

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15.1 Introduction

The use silver nanoparticles (AgNPs) has become common in daily products employed in modern society. These nanoparticles (NPs) can be founded in wound dressings, food pack, medical devices and even in textiles industry [1–3]. Surprisingly, in a list of 1015 products present on the markets containing NPs, 259 of them contain AgNPs [4]. Therefore, due to their unique properties, these NPs have been entered in our houses without ask license exposing us to an unknown threat. Moreover, their physical, chemical and optical properties are being highly studied and exploited by researchers across the world for different purposes [5, 6]. However, the utilization of antibacterial potential of silver is not recent but it goes back to Hippocrates, the father of medicine, who used the silver to treat ulcers and spread the biocidal potential of this metal to the west civilization [7].

The AgNPs are formed by agglomerates of silver atoms ranging from 1 to 100 nm which are metallically bonded. Owing to their nano-size, these NPs present large surface area ratio and high reactivity being sensitive to oxygen [5]. The study of the relationship between silver nanotechnology's and its possible toxic effects to man health is relatively new and some studies have shown that these NPs can be toxic to mammalian cells [8–10].

In the sixteenth century, Paracelsus, known as the father of toxicology stated that all substances are potentially harmful, what makes something into a poison is just the dose. In this context, the elucidation of dose response of cytotoxic effect induced by AgNPs will allow their safe use for a multitude of industrial applications, as well as their employment for therapeutic purposes [11]. In addition to the concentration, it is important to determine the relationship between size, shape and toxicity, so that the NPs are synthesized with the desired properties.

Therefore, this chapter will explore the aspects related to the toxicity of these NPs discussing their use in the treatment of water and their consequent release into aquatic ecosystems, the influence of interaction with organic matter on their toxicity and the mechanisms of action of these NPs in cells and various organisms. The question of AgNPs safe use can be compared with decisions made from coin flipping. Initially used by Roman soldiers and known as "navia aut caput", the coin flipping offers only two different possibilities, heads or tails, which represent totally different results obtained from the same coin.

15.2 AgNP Toxicity in Aquatic Environments

Water is an essential need for life and the access to potable water is considered one of the most basic humanitarian goals. In view of this fact, the use of technologies including filtration, ultraviolet radiation, chemical treatment and desalination has been well established since the ancient civilizations [12–14]. As previously mentioned, NPs and especially AgNPs are present in a multitude of daily products. Thus, cleaning or disposal of these objects may result in the release of these NPs into the environment. Moreover, nanomaterials have become very useful in water treatment because of their different properties like high reactivity, high surface area, and high adsorption when compared to materials in macroscopic scale. Interestingly, one of the nanomaterials most used in water treatment are AgNPs. Among the various biocide mechanisms of AgNPs, attention is directed to their ability to attach to cell membrane and penetrate bacterias compromising their respiratory chain and cell division [15]. Another effect of AgNPs on bacteria is the Ag^+ ion release. These ions interact with thiol groups resulting in enzymatic damage and preventing DNA replication [13]. Due to this bactericidal effect of AgNPs, there is a large variety of materials which employs these NPs for water disinfection (Table 15.1).

Membranes provide a physical barrier for undesirable matter based on their size. They provide a high level of automation, require less land and chemical use, and allows flexible design, besides the possibility of addition of components that improve the removal of pathogenic microor-

Table 15.1 Different AgNP coated materials used in water treatment

Material coated with AgNPs	Effectiveness	References
Membranes	Effective elimination of *E. coli*, *P. aeruginosa*, *B. subtilis*, *and S. aureus*	[14]
Foam	Effective elimination of *Escherichia coli*	[15]
Filter	Completely effective against *Escherichia coli*	[16]
Porous ceramic	Effective elimination of *Escherichia coli*, output count was zero	[17]
Woven fabric	100% efficient in elimination of *Escherichia coli*	[18]
Paper sheet	Significant biocidal action against *Escherichia coli* and *Enterococcus faecalis*	[19]

ganisms [13]. The addition of AgNPs in membranes is very common; these NPs can be anchored on a polymer (usually methacrylic acid copolymer due to its unique characteristics such as insolubility, mechanical strength, and macroporous nature). This method was effective to eliminate *E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. aureus* [14] (Fig. 15.1).

AgNPs can be coated on foams and be used as antibacterial water filters through a non-toxic and cheap process. The polyurethane coated with AgNPs resists to storage, washing, and drying without AgNPs release and no bacterium was detected in the output water when the input water had a bacterial load of $1 \times 10^5 - 1 \times 10^6$ CFU mL^{-1} (colony-forming units' mL^{-1}). Results from standard test such as "inhibition zone" and "test tube" are in agreement with WHO requirements for drinking water [15].

Another material used in water treatment is ceramics due to their long lifetime and resistance to high temperature, pressure and corrosive solutions. The addition of AgNPs makes them very useful to kill bacteria. At a flow rate of 0.01 L min^{-1}, the output count of *E. coli* was zero when the input water had a bacterial load of $\sim 10^5$ CFU mL^{-1}, proving the high efficient of this material in the water treatment [17]. The large utilization of different materials for water disinfection calls attention about the lifetime of them. Haider and colleagues [20] have studied aminated polyethersulfone-silver nanoparticles (AgNPs-APES) composite membranes and reported the release of ionic silver after 12 days [20]. Therefore, in order to ensure the safety use of these membranes and not exceed the allowed concentration for silver in drinking waters, it is important to find mechanisms to prevent the release of silver during these disinfection processes [21].

The release of silver (as NPs or ions) can affect the human health and the environment. AgNPs impregnated in coal of water filters used in home treatment devices can represent risks to human health due to the Ag^+ release in the purified water [14]. Furthermore, the sewage sludge resulting from water treatment is often used as fertilizer for agricultural soils, thus, AgNPs can be leached to aquatic systems and enter in food web by the primary producers. The first toxic effects on primary producers like algae are the decrease in chlorophyll content, damage in photosynthesis, increase of ROS (Reactive Species of Oxygen) and lipid peroxidation [22–24]. Secondary organisms like crustaceans (*Daphnia magna*) can be affected by AgNPs in water or by ingestion of primary producers and among these effects can be cited the abnormal swimming and decrease of reproduction [25, 26]. Malformations in embryos of zebrafish due to exposure to AgNPs have also been reported [10]. In this context, some studies have demonstrated that these NPs can be toxic to algae [27], fish [28], snails [29] and plants [30]. On the other hand, other analyzes have shown that the prolonged exposure to these NPs cannot be very harmful to the aquatic ecosystem. Jiang and coworkers [31], for example, demonstrated that the chronic exposure to AgNPs or $AgNO_3$ during 90 days does not significantly affect the phytoplankton biomass and the diversity of aquatic plants and animals [31].

AgNP toxicity is related to their size, shape and load, so the synthesis methods used in their manufacture must be controlled to obtain the desired properties. Moreover, the subsequent NP characterization analyzes by means of dynamic

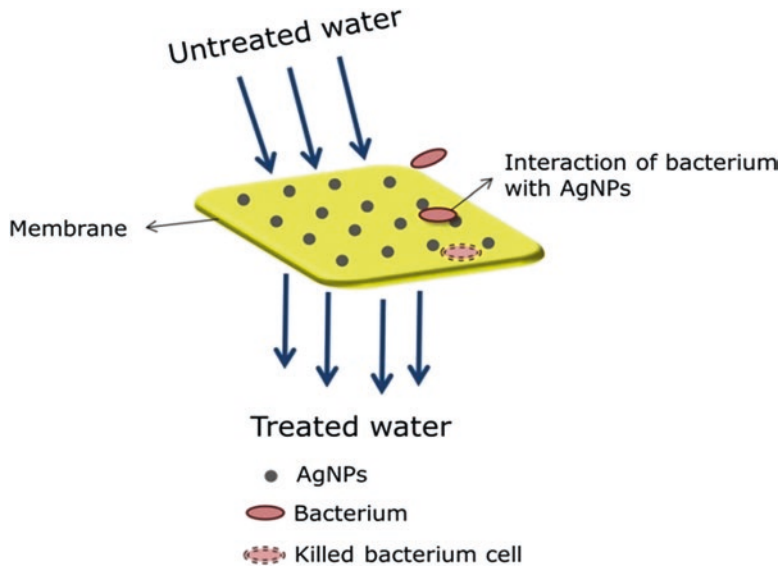

Fig. 15.1 Schematic representation of a membrane coated with AgNPs and its effects against bacteria

light scattering (DLS), scanning and transmission electron microscopy allow the comparison among different researches, thus elucidating the AgNP toxicity and helping to establish safe standards for their utilization. In this context, strategies comparing different sizes of AgNPs are extremely relevant because they elucidate the relation between size and toxicity. However, most of studies exploring AgNP toxicity disregard a very important step in determining the real risk offered by these NPs, which is the simulation of interactions between organic compounds with AgNPs after their releasing into the environment. These compounds may modulate or even inactivate the toxicity of AgNPs, demonstrating that the real damage caused by these NPs may be overestimated in in vitro and in vivo studies which do not consider these interactions.

15.3 AgNP Interaction with Natural Organic Matter (NOM)

The aquatic ecosystem is the most easily to be contaminated with NPs and their interactions and behavior with water, NPs and natural organic matter (NOM) are extremely important in studies of their toxic behavior in this environment. The NOM consists of a large variety of organic molecules that is referred as humic substances and is rich in humic and fulvic acids. These substances have great importance in binding metals, and because of this, can affect the transport and stability of metal NPs [32, 33]. When NOM is in contact with NPs, it can modify NPs proprieties by adsorption onto surface forming a coat [34].

When AgNPs enter into aquatic environment, they can interact with NOM. The adsorption of NOM by AgNPs depends on two factors: (i) the composition of NOM and (ii) the capping agent of AgNPs. If the amount of sulfur and nitrogen is higher in NOM, the adsorption increases. On the other hand, if the bonds between the capping agent and AgNPs were lost the NOM can bind to AgNPs, stabilizing them [35]. Low concentrations of NOM increase NP stability; however, high concentrations of NOM can stimulate the agglomeration of these NPs [36, 37]. The stabilization of AgNPs by NOM occurs by its adsorption on AgNPs´ surface preventing their agglomeration.

In the dissolution of AgNPs, Ag^+ species can be released; these Ag^+ ions are well known to be toxic to the environment because they induce oxidative stress [38]. However, the NOM adsorption on AgNPs affects their dissolution proprieties reducing the Ag^+ release in a dose-dependent manner. This ion releasing decreases by different mechanisms like the adsorption of NOM blocks,

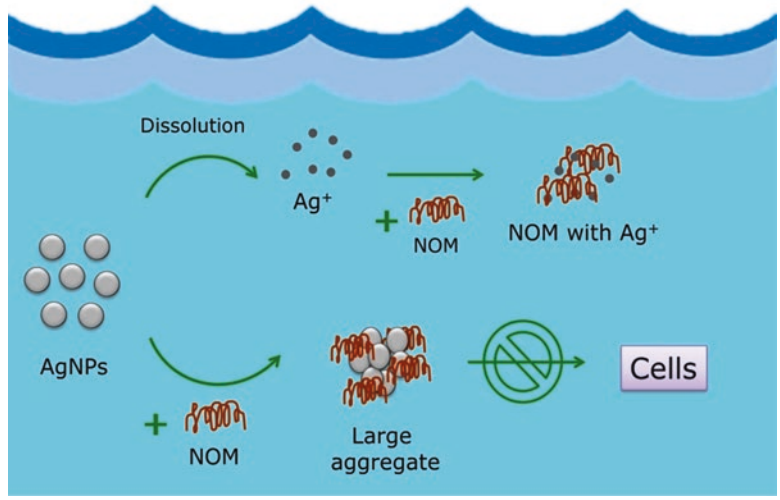

Fig. 15.2 Schematic representation of the interactions among AgNPs, NOM, and Ag^+ ions

the oxidation of AgNPs sites and the reversible reaction of Ag^+ formation from Ag^0 due to the humic/fulvic acids acting as reducing agents [39]. In addition, even when release of Ag^+ ions occurs, NOM can bind to these ions decreasing its toxicity [37, 40]. Interestingly, NOM binding to Ag^+ ions can reduce, coat, and stabilize these ions forming AgNPs naturally [41].

Due to the binding proprieties of NOM, the concentration of free Ag^+ ions can decrease, thus their toxicity also decrease [34, 35, 40]. More specifically, when NOM is in high concentrations, it stimulates the formation of AgNPs agglomeration; these agglomerates are large and cannot enter in cell membrane, thus decreasing the AgNPs toxicity, as represented in Fig. 15.2.

The decrease of toxicity of AgNPs coated with NOM is also related to their lower bioavailability. The formation of larger agglomerates with high molecular weight favors their removal into sediments, decreasing their bioavailability [41]. Besides all these factors, the composition of NOM is also an important factor in the toxicity of AgNPs: a higher amount of sulphur reduces the dissolution of AgNPs, and consequently the concentration of Ag^+ ions decrease and also their toxicity [42].

The effects caused by AgNPs in the environment are closely related to the interactions occurring in the ecosystem; and the organic matter is important in this context. These associations have impacts on the NP toxicity, decreasing the bioavailability and concentration of Ag^+ ions. Nevertheless, more studies exploring the interaction between AgNPs and organic matter should be done to unveil the real risk offered by the exposure to these NPs.

Some studies have also shown that the interaction between NPs and mammalian cells can cause lesions in the genetic material [9, 43, 44]. Our group used the micronucleus test and comet assay to demonstrate that AgNPs can induce chromosomal breaks and genotoxic damage [9]. Furthermore, high NP concentrations can be cytotoxic, causing cell death by various mechanisms such as apoptosis, necrosis and autophagy. Although numerous studies [9, 43–45] have confirmed the direct and indirect cyto- and genotoxic potential of AgNPs in vitro and in vivo, the mechanism of action of these NPs is still uncertain. However, increasing evidence has corroborated the Trojan-horse mechanism as responsible for AgNP toxicity [46, 47].

15.4 AgNPs and the Trojan Horse Mechanism

The antimicrobial potential of silver materials is related to Ag^+ ion release after the interaction with oxygen. AgNPs in aqueous solution release Ag^+ ions, which are biologically active and can mediate the bactericidal effect [6] as well as lead

to significant cytotoxicity in mammalian cells [48, 48]. Studies demonstrate that Ag^+ ions can interact with cytoplasmic components and nucleic acids, resulting in the inhibition of respiratory chain enzymes, and interfering in membrane permeability [5, 49]. Thereunto, an effective way of quantifying AgNP's toxicity can be the measurement of AgNPs/Ag^+ ratio in the intra and extra cellular medium [46]. The strategies used to separate AgNPs from Ag^+ ions are centrifugation, ultrafiltration, and cloud point extraction. To quantify each of these components, the atomic adsorption spectroscopy or mass spectroscopy techniques have been used [46, 47, 50–52]. Wang and colleagues [47], for example, separated AgNPs and Ag^+ ions into erythroid cells (MEL) from mice by means of a cloud-point extraction and found AgNPs (82.1%) and Ag^+ ions (17.9%) together inside cells, which suggest the occurrence of a Trojan-horse type mechanism [47]. However, it is still necessary to compare the internalization rate of AgNPs and Ag^+ in order to determine if ionization is really occurring in the intracellular environment [46].

However, one study comparing AgNPs with others silver solutions revealed that these NPs have a greater antibacterial potential than free Ag^+ independent of elution [52]. Recent evidences show that AgNPs can produce many reactive oxygen species (ROS) including superoxide-radical (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), and singlet molecular oxygen ($^1O_2$) [53]. Furthermore, H_2O_2 can react with internalized AgNPs to form more Ag^+ ions, thus, the Trojan-horse mechanism is a chain reaction which results in an increasing release of Ag^+ [46]. Inside cells, Ag^+ can react with other ions such as Cl^- and S^{2-}, forming AgCl and AgS_2, respectively [54, 55]. Moreover, other compounds such as Ag-cysteine and Ag_2O can be formed (Fig. 15.3). Oxidative stress can induce DNA and protein damage and lipid peroxidation [56], which partially explains how AgNPs can present antibacterial effects and are potential toxic to humans [57].

Moreover, Park and colleagues [58] reported that AgNPs can promote various genetic and physiological modifications, such as increased expression of matrix metalloproteinases and decreased intracellular glutathione expression [58].

15.5 AgNPs as Nanocarriers (NC)

Nowadays, the flexibility in NP synthesis allows the production of nanocarriers (NC) with some tunable properties like solubility, particle size, circulation half-life and degradation. These nanosystems can be engineered to target organelles, cells or tissues in a specific way reducing these previously mentioned side effects [11].

The cellular uptake of NCs can occur through a passive translocation across the bilayer membrane or by physicochemical techniques such as electroporation. Due to NP large surface area and curvature relative to their volume, cells activate the endocytosis process for their uptake [11]. This cellular uptake process can be used as a strategic pathway to deliver drugs in specific cells. Thus, drugs can be loaded with NPs and target moieties on the surface which will act against receptors without affect normal cells. Many receptors have been discovered for cancer drug targeting, the most commonly used is the folic acid [59]. NPs can spread over different tumors regions by blood vessels and then to interstitial space until arriving cancer cells, eradicating them [60, 61].

AgNPs are potential anticancer agents and some studies using biologically synthesis methods prove this potential [62]. Cytotoxicity studies of AgNPs using *Melia dubia* extracts against human breast cancer cells showed that low concentrations of these NPs were able eliminate 50% of cancer cells [63]. Moreover, AgNPs synthesized using *Malus domestica* (apple) extract showed significant cytotoxic effects against MCF-7 breast cancer cells [64]. Another study that used AgNPs engineered by *Nonotus obliquus* (Chaga mushroom) extract showed effective antiproliferative activity toward A549 human lung cancer and human breast cancer cells (MCF-7). These studies, demonstrate that AgNPs produced by green synthesis methods possess high cytotoxic activity against cancer cells which suggests

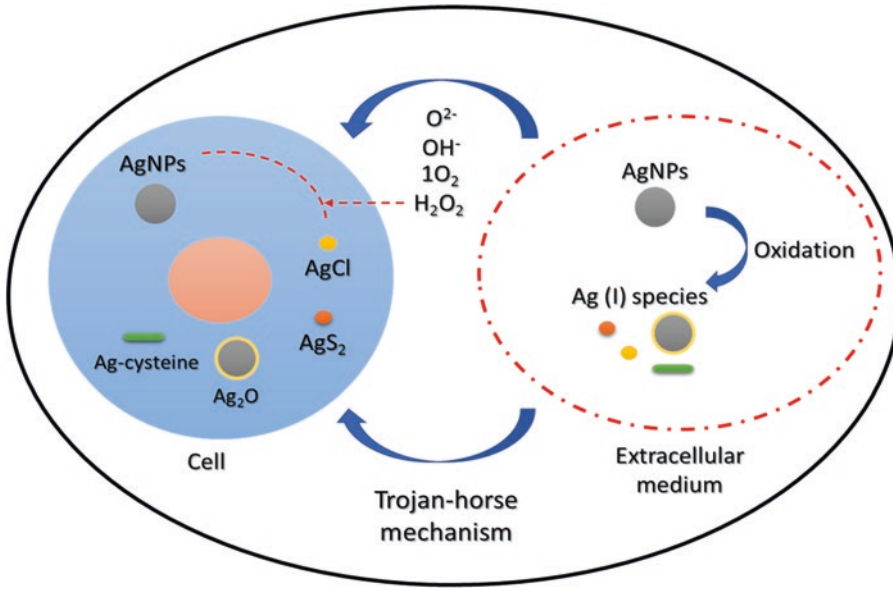

Fig. 15.3 Illustrative scheme of the "Trojan-horse" mechanism. AgNPs are internalized by cells and then are oxidized forming Ag^+ which can react with free ions such as Cl^-, S^{2-} and O^{2-} or cysteine in the intracellular medium. Ag^+ can also induce the formation of ROS inside cells (Modified from [46])

the potential therapeutic use of these NPs as a "Trojan Horse" strategy against cancer [65].

Recently, Pang and coworkers [66] used macrophages cells as "Trojan-horses" to carry drug-loading NPs which could pass through cellular barriers and offload them into brain tumor sites. Free anticancer drugs encapsulated into cells could cause damage to the carrier itself before arriving at tumor sites suppressing the functions of cells as transporters. Therefore, the researchers encapsulated anticancer drugs into NPs to reduce the damage of the drug to cell carriers. In this investigation, nanodrugs were encapsulated into patient macrophages, then these macrophages loaded with drugs-NPs were transferred back into the patient to achieve improved efficacy and to reduce immune responses [66].

Wang and colleagues [47] showed that AgNPs reduced the efficiency of cell transcription due to the direct binding of silver to RNA polymerase. The drugs that inhibit microbial or viral RNA polymerase activity have been used against invading pathogens [47]. Therefore, this work is an interesting example about how Trojan-horse mechanism can be used for medicine research. Evidence for AgNP toxicity through this mechanism was also found in mouse macrophages (RAW264.7) and in human bronchial epithelial cells (BEAS-2B) [58, 67, 68].

On the other hand, NPs can be captured by central nervous system through microglia and astrocytes cells, representing a threat to neuronal cells [69]. In vivo studies have been shown that AgNPs can accumulate on the developing brain, leading to developmental dysmorphologies [70]. The potential neurotoxicity of AgNPs is also related to ROS induced by NPs which may be associated with neurodegenerative disorders [46]. Nevertheless, studies evaluating the implications and applications of AgNPs in biological systems are still recent and how this NPs influence people health remains unanswered.

15.6 AgNP Cytotoxicity: Apoptosis, Necrosis and Autophagy

Cells which are unnecessary for the organism commit suicide by activating an intracellular programmed death known as apoptosis. This process is morphologically characterized by pyknosis

(deep staining of nuclear mass), nuclear fragmentation, and formation of condensed cell bodies (apoptotic bodies). On the other hand, necrosis process occurs when cell suffer an unexpected and accidental damage. Therefore, toxic chemical or physical events like toxins and radiation exposure can result in an electron-lucent cytoplasm [71, 72].

In vitro studies have shown that AgNPs can be cytotoxic to human cells. Some researchers reported that AgNPs decrease the viability and proliferation of keratinocytes and human liver cells [73, 74]. The researchers discovered that AgNPs interfere in cell cycle and lead to increase of apoptosis both in brain tumor cells and normal fibroblasts [75]. However, the mechanisms related with AgNP cytotoxicity are still not totally clear.

As described in the last session, the oxidative stress mechanism mediated by AgNPs in cells is caused by an imbalance between oxidants and antioxidants and resulted in damage to cells organelles such as mitochondria and endoplasmic reticulum, [76–78] which activates apoptosis in mammalian cells [79–82].

ROS are mainly generated in the mitochondria [83], but some studies show that other cell organelles like endoplasmic reticulum (ER) also respond to oxidative stress playing an important role in the outcomes activated by AgNPs [84, 85]. The endoplasmic reticulum is involved in protein folding and assembly, lipid biosynthesis, vesicular traffic, and cellular calcium storage. This organelle is sensitive to alterations in homeostasis; thus, any change in cell metabolism can compromise its function, inducing cellular damage and apoptosis [86].

ER related changes such as inhibition of protein glycosylation, reduction in disulfide bond formation, calcium depletion from the ER lumen, impairment of protein transport from the ER to Golgi apparatus and expression of misfolded proteins may causes proteotoxicity in this organelle causing an endoplasmic reticulum stress [87–89]. Considering that this organelle is essential for cell survival, changes in ER function interfere in cell apoptosis and some studies already have reported the importance of ER in apoptotic process [90].

It is known that the toxic effect of AgNPs depends on their size and the coating material [91–93]. Liu and colleagues [92], for example, demonstrated that small AgNPs (3–4 and 5–7 nm) were more toxic than 10–40 nm NPs to mouse cells [92]. However, our group demonstrated that 100 nm AgNPs tend to be more toxic than their smaller counterparts (10 nm) [9]. Therefore, although there is no consensus about the relationship between size and toxicity in these NPs; the NP diameter is directly related with the biocide potential of AgNPs. On the other hand, Gliga and co-workers [91] demonstrated that citrate coating affect NP toxicity with the exception of 10 nm AgNPs [91]. Furthermore, the cytotoxic effects of AgNPs may cause different responses depending on the cell type. Kim and co-workers [94], for example, reported that AgNP cytotoxicity stimulated apoptosis in osteoblastic cells; otherwise it induced necrosis in adrenal medulla cells in mice [94]. These opposite effects can be activated in dependence of molecular mechanism differentially expressed in cells from diverse origins which can affect proactive pathways in those cells.

Asare and colleagues [95], for example, reported on in vitro study that AgNPs can caused DNA damage, apoptosis, necrosis and proliferation decrease in murine primary testicular cells as well as tumor cells [43, 95]. In this context, Li and coworkers [96] evaluated the cytotoxicity of AgNPs decorated by polyethylenimine (PEI) and paclitaxel (PTX) (Ag@ PEI™ PTX) in HepG2 cancer cells. Induction of apoptosis in these cells after exposure to Ag@PEI & gt; PTX was verified due to DNA fragmentation, depletion of mitochondrial membrane potential, activation of caspase 3 and increase in cell population in sub-G1 phase of cell cycle [96]. Assays using 7-AAD and Annexin-V dyes demonstrated that AgNPs have the potential to induce cell necrosis or accidental cell death. This induction has been shown to be related with size and time of exposure to AgNPs [97].

Autophagy is a degradation process of toxic proteins and damaged organelles in which portions of the cytoplasm are stocked in autophagosomes and then are fused with lysosomes forming autolysosomes. Posteriorly, the autolysosomes content is degraded by lysosomal hydrolases and recycled for energy utilization [98]. The autophagy process is mainly characterized as a survival mechanism from different environmental stresses such as AgNP exposure. It has been proposed that AgNPs can induce this type of cell response by interfering in the ubiquitination process. These NPs would be able to promote the increase of enzyme levels that participate in the ubiquitination and avoid the biological reactivity of ubiquitin [99].

NP-induced autophagy has been considered as a potential molecular target for NPs based chemotherapy [100–102]. The NP-activated autophagy has been associated with inflammation, oxidative stress, and induction of apoptosis [103]. Until now, studies involving autophagy and apoptosis after NPs exposure suggest that these NPs can cause irreversible damage to cell [104].

Considering the increasing use of NPs in many manufactured products, some researchers are also evaluating the possible effects that the interaction between AgNPs and others nanometals may cause to cells. In recently studies, the associations of AgNPs and metal ions of cadmium and mercury, which are found in various environmental contaminants (e.g. battery fluid, fertilizers, paints, plastic stabilizers, coal combustion, and seed treatment), were more toxic than AgNPs [105, 106]. The association caused a decrease in cell viability and changes the cell death type from apoptosis to necrosis [107]. Thus, these results suggest that there is still much to understand about NP interactions with other nanomaterials and how this may influence our lives.

15.7 Conclusion

Nowadays, AgNPs are the most widely used NPs in the industry because of their peculiar biocide features. The applications of these features to industrial and therapeutic purposes have been brought enormous benefits to our society. However, the employment of these NPs still runs into limitations mainly because of lack of standardization of size and shape and the absence of dose-dependent toxicity elucidation. Added to these obstacles is the scarcity of studies measuring the toxicity of these NPs after their interaction with organic matter and their intracellular mechanisms of action. Thus, further studies should explore these issues to potentiate the applications of the unique AgNPs properties.

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