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Silver nanoparticles for delivery purposes

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15.1 Synthesis of silver nanoparticles

The synthesis of metal nanoparticles is currently a highly active area of research. Several methods have been developed for the synthesis of these materials. Techniques for synthesizing nanoparticles can be divided into solid-, liquid-, and gaseous-phase processes [1].

Due to the size of nanoscale materials, their behavior is remarkable, compared with their macroscopic counterpart. The properties of these materials are primarily influenced by the increase in the surface-to-volume ratio, which results in an increase in the total contact/active surface. The solubility or the reactivity is superior to those of the same materials in the form of larger particles [2]. Consequently the development of techniques that can lead to the production of nanoparticles with adequate size and shape and controlled polydispersity is pivotal.

Nanocrystalline materials can be synthesized by either clustering atoms/molecules/groups of atoms (bottom-up approaches) or decomposing large-sized materials to smaller dimensions (top-down approaches). During top-down synthesis, nanoparticles are obtained by reducing the size of macroscopic systems to nanoscale. The reduction in particle size can be achieved by various physical or chemical procedures when applying a source of energy, which may be mechanical, chemical, or thermal [3, 4].

As far as mechanical approaches are concerned, there are various types of milling equipment used to mix, alloy, or reduce in particle size. The *mechanical milling* processes may involve high local temperatures and pressures (> 1000°C and GPa-level pressures), which is why these types of processes can be considered to be mechanochemical synthesis. The aim of milling is to reduce larger particle sizes to nanoparticles, with the possibility of generating new phases and surface properties, as function of the milling parameters [5]. Generally, using top-down approaches (e.g., milling) makes it relatively difficult to obtain precise control of the size and shape of the nanoparticles; however, it is a quick and adequate variant for widespread use in the nanoindustry [6].

To improve the process of nanoparticle synthesis by mechanical milling, surfactants are generally used, which help to obtain particles with more precise sizes and superior characteristics. Surfactants are materials that may exhibit both hydrophobic and hydrophilic properties. A major classification of these is defined based on the type of surface charge, namely, anionic, cationic, amphoteric, and ionic surfactants [5]. The main problem posed by nanomaterials obtained by mechanical means is their irregular

shape and the presence of defects in the crystalline network, as well as the partially amorphous state of the powder. Moreover the final product may be contaminated with impurities due to the grinding media [7, 8].

Another means of top-down synthesis is *laser ablation*. This technique is based on the principle of optical conversion in thermal energy due to electronic excitation. By using high energy lasers, an increased absorption of light on the surface of the target material is caused, where rapid temperature rise occurs and chemical bonds from the surface of the target material are destroyed, allowing the target material to evaporate. Particle synthesis is accomplished by condensation of the removed (evaporated) material from the target surface, leading to the formation of nanoparticles in a liquid [8a]. It is understandable that the processing parameters greatly affect the characteristics of the laser-ablated nanoparticles. Sportelli et al. are reporting that exceptionally stable silver nanoparticles were prepared by LASiS using isopropanol as ablation medium, instead of water. The nanoparticles synthesized using a pulse energy of 46.5 mJ exhibited stability over time (up to 90 days), without any significant flocculation. The reason for this stability might be related to the decomposition of isopropanol at high temperatures (generated by the laser beam), thus generating organic stabilizing layers, which cover the nanoparticles [9]. Furthermore, by applying several stages of laser irradiation, firstly, to the solid target and, secondly, to the solution obtained in the first stage, one could obtain reduced-size silver nanoparticles, as reported by Fernández-Arias et al., with increased inhibitory effects against *Staphylococcus aureus*, clearly related to the improved size and shape of the nanoparticles, caused by the reirradiation stage [10]. Laser ablation could be used as a single-step process to deposit silver nanoparticles onto substrates, as reported by Boutinguiza et al. In this case the spherical shape AgNPs, with mean diameter below 20 nm, were proposed as potential antibacterial agents against *Lactobacillus salivarius*, to be further used in dental implantology [11].

The *sonofragmentation* process involves breaking the particles into nanoscale fragments by applying high-power ultrasounds. In the case of sonofragmentation, detachment occurs due to the interaction between the particles and the shock waves, unlike the usual ultrasonic milling, where the particles are mainly milled and separated by collision between the particles [12, 13]. Ruixuan Gao et al. have used ultrasonication applied to metal nanowires to generate monodispersed metal nanoparticles. They applied sonofragmentation on several types of materials from Ge, to TiO₂, to Ag. For the synthesis of Ag nanoparticles, commercially available nanowires of Ag with a diameter of about 20 nm were used. Following the sonofragmentation process the obtained nanoparticles had dimensions smaller than 4 nm [14].

Similar to laser ablation (where the nanoparticles are obtained due to the effect of a laser beam on a solid target), *sputtering* is a method of vaporizing materials on a solid surface by bombarding it with energetic ions of a plasma, causing an ejection of atoms and groups of atoms, followed by condensation into nanoparticles [15]. Magnetron sputtering can be used to synthesize metallic nanoparticles, and their size can be controlled with precision. With a constant sputtering time and deposition time, the size of the Ag nanoparticles is inversely proportional to the target-substrate distance. These Ag nanoparticles have important properties for potential applications both in the catalyst and sensor industry and in diagnostics [16]. Wender and colleagues reported the

formation of colloidal silver nanoparticles after sputtering and condensation in castor oil, canola, and capric-caprylic triglyceride oil. Nanoparticles that showed smaller dimensions and a uniform size distribution were those obtained with increased discharge voltage [17]. Several other physical and chemical methods have been used to produce nanoparticles, such as ultraviolet irradiation, aerosol technologies, lithography, ultrasonic fields, and photochemical reduction techniques, although they remain expensive and involve the use of hazardous chemicals [18].

In “bottom-up” synthesis processes, the individual manipulation of atoms and molecules through self-assembly processes leads to the formation of nanostructures. The precursor is usually a liquid or gas that is ionized, dissociated, sublimed or evaporated, and then condensed to form amorphous or crystalline nanoparticles [4, 19, 20]. The main advantages of bottom-up techniques consist of a homogeneous chemical composition, a low particle size variation, or the number of nanoparticle surface defects, considerably lower compared with top-down approaches [4].

Chemical reduction is the most commonly used method of synthesis of nanoparticles due to its low difficulty. Through this bottom-up approach, controlling the growth of metallic nanoparticles with a narrow distribution in diameter is a viable goal. It is well known that metal nanoparticles can be produced through this process at low cost and high yield [20, 21]. To understand the principle of the method, the principle underlying the chemical synthesis of metal nanoparticles will be described. The process requires three components, namely, metal precursors, reducing agents, and stabilizing agents [22]. The formation of the colloidal solution, which contains nanoparticles reduced from metallic salts, involves two main steps: the nucleation/germination step and the subsequent growth of the crystals. It has been demonstrated that the size and form of synthesized nanoparticles are strongly dependent on these stages [23, 24]. For the synthesis of uniformly dispersed nanoparticles, it is necessary that the formation of all nuclei is simultaneous. The formation of crystals can be controlled by adjusting the reaction parameters such as temperature, pH of the solution, precursors, reducing agents (ethylene glycol and glucose), and stabilizing agents (PVA and PVP) involved in the synthesis. Moreover a short nucleation burst, followed by slow controlled growth, is essential to produce monodisperse nanoparticles [25, 26]. When discussing chemical reduction the synthesis process is divided in two stages: In the first stage a strong reducing agent is used to produce small particles, while in the second stage, these small particles are grown by further reduction with a weaker reducing agent.

In recent years, *biological synthesis (biosynthesis)* has emerged as an attractive alternative to traditional nanoparticle production methods. Biosynthesis involves ecological approaches based on green methods using single-cell and multicellular biological entities, such as bacteria, actinomycetes, fungi, plants, yeasts, or plant extracts [27]. In addition to the use of microbes and plants, green methods of synthesis currently include different approaches through the use of biological materials like honey, starch, or ascorbic acid. They have been used so far to synthesize gold, silver, palladium, carbon, and platinum nanoparticles [28]. Although microorganisms and plant extracts can be used to synthesize metal nanoparticles, it is very important that the process be optimized to produce homogeneous nanoparticles of similar size and shape. This can be done by adjusting the control parameters such as the precursor concentration, the

mixing ratio between the biological extract and the metal salt, pH value, temperature, incubation time, nutrient media composition, and aeration [29].

Plants could be considered a more ecological way for the biological synthesis of metal nanoparticles. They have potential in the accumulation and biological reduction of metal ions. Plant extracts contain bioactive alkaloids, phenolic acids, polyphenols, proteins, sugars, and terpenoids that play an important role in the initial reduction of metal ions and furthermore in their stabilization. The variation in the composition and concentration of these active biomolecules between different plants and their subsequent interaction with metal ions contributes to the diversity of nanoparticle sizes and shapes that can be obtained [27, 29].

Natural products or those derived from natural products, such as extracts from several plants or parts of plants, tea, coffee, bananas, and plain amino acids, as well as wine, table sugar, and glucose, have been used as reducing agents [29]. Recent experiments have also revealed the reduction potential of leaf extracts, seed extracts, root extracts, bulbs, and plant latex, which are used to synthesize gold, silver, and palladium nanoparticles [28].

Bioreduction consists in the chemical reduction of metal ions in more stable forms. Many organisms have this ability, where the reduction of a metal ion is coupled with the oxidation of an enzyme. This results in stable and inert metal nanoparticles [30]. The most common location of nanoparticle biosynthesis is that of biological cells and their cell membrane. Biosynthesis is the phenomenon that occurs through biological or enzymatic reaction [31]. There are two types of biosynthesis, depending on where the process takes place, that is, intra- or extracellular synthesis. Intracellular synthesis occurs in the cell, while extracellular synthesis occurs due to cell-secreted enzymes [32].

The ability of bacteria to synthesize inorganic nanoparticles is well known and explored. The first bacteria that have been shown to have the ability to produce silver nanocrystals belong to the strain *Pseudomonas stutzeri* A259 [33].

Hereinafter the procedure to obtain silver nanoparticles using *Bacillus amyloliquefaciens* and *B. subtilis* will be briefly presented. The bacteria were grown in the following solid medium: 1-g/L yeast extract; 18-g/L agar-agar; 5-g/L sodium nitrate; and 0.2-g/L glucose, which was firstly sterilized at 128°C in 100-mL volume of distilled water. The cultures were further inoculated on the solid medium petri dishes, with 1 μ L of each bacterium and incubated at 33°C for 48 h. To synthesize the silver nanoparticles, 1 μ L of bacterial strains was freshly inoculated into conical flasks containing 100 mL of liquid medium (0.6-g/L yeast extract, 1-g/L sodium nitrate, and 3-g/L glucose) at 33°C for 48 h. After this incubation period the cultures were centrifuged at 4000 rpm for 30 min. Ten milliliter of the supernatant was mixed with 90 mL of the precursor (1-mM aqueous AgNO₃ solution). The steps are shown schematically in Fig. 15.1.

The samples were incubated for 48 h at 33°C and 150 rpm. Furthermore the solution containing the biosynthesized AgNPs was centrifuged. The collected material was washed with 25 mL of distilled water and dried at 80°C until the liquid was evaporated. The starting point for the formation of silver nanoparticles was observed after 6 h, when the color of the aqueous solution began to change from pale yellow

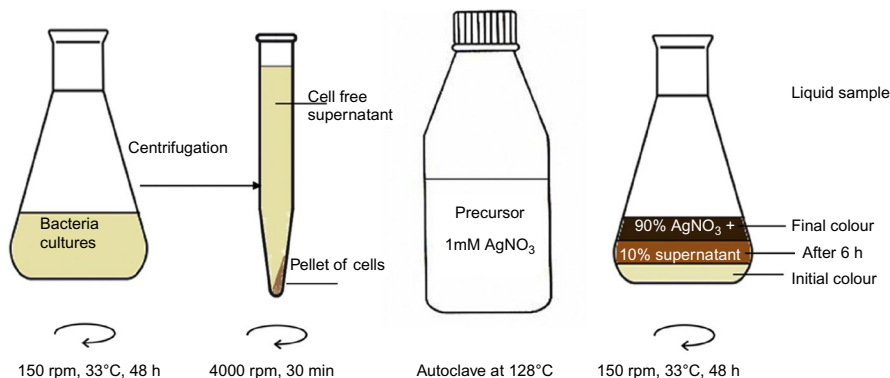


Fig. 15.1 The biosynthesis steps used to obtain AgNPs, *Bacillus amyloliquefaciens* and *B. subtilis* aided.

to light brown, due to the surface plasmon resonance phenomenon [34]. After 48 h of incubation, the final color of the samples was changed to brown, thus signaling the extracellular synthesis of silver nanoparticles. The reduction of silver nitrate could be produced by the constituents of the cell supernatant. Peptides or proteins may be responsible for the reduction of Ag^+ ions and the subsequent formation of silver nanoparticles. The synthesis of silver nanoparticles can be mediated by the alpha-amylase enzyme, produced by the *Bacillus* species [35]. Further results concerning the structural and morphological features of the synthesized silver nanoparticles and results related to their antibacterial capacity and synergistic effect when combined with fluconazole and ciprofloxacin, against several bacteria strains and fungi, can be found elsewhere [36].

The use of fungi in nanoparticle synthesis and micosynthesis displays advantages compared with other organisms, especially due to their relatively easy isolation and their capacity to generate higher protein concentrations, or enzymes that help reduce metal ions [29, 37]. Eukaryotic organisms, such as fungi, have been thoroughly investigated for their ability to form nanoparticles. In a large study involving nearly 200 different genres, Sastry et al. found that fungi are excellent candidates for the synthesis of metal nanoparticles and metal sulfides. The rapid reduction of metal ions by two different types of fungi, *Verticillium* sp. and *Fusarium oxysporum*, exposed to aqueous solutions of gold and silver ions was reported [38].

Of all eukaryotes, yeasts are probably the most studied and applied in bioprocesses. Moreover, their potential to produce semiconductor nanoparticles is well known and investigated. Although yeasts are known to predominantly produce nanoparticles intracellularly, recent studies have revealed the extracellular synthesis of silver nanoparticles using the silver-tolerant yeast strain MKY3 [39].

The identification of prokaryotic microorganisms for the synthesis of gold nanoparticles was reported by Ahmad and his collaborators, where actinomycetes of the *Rhodococcus* species helped synthesize nanoparticles with well-defined dimensions and good monodispersity. Similar results were also obtained by extracellular synthesis using *Thermomonospora* sp. [28, 40, 41].

A combination of the previously mentioned synthesis processes could be employed to further synthesize silver nanoparticles, with the main advantage of significantly shortening the synthesis period, as reported by Matej Baláz et al., where by using a biomechanicochemical approach and thus combining mechanochemistry (ball milling) and green synthesis, silver nanoparticles (AgNPs) with antibacterial activity were successfully synthesized [42].

For an extensive coverage of the chemical and biological synthesis of metal nanoparticles, there are several recent reviews available, which can help the reader to further explore the subject [4, 43–50].

15.2 Applications of silver nanoparticles

15.2.1 AgNPs as delivery systems in cancer therapy

Cancer is the second major problem of global mortality, being responsible for an estimated 9.6 million deaths in 2018. According to the World Health Organization, the most common causes of cancer-related deaths are lung cancers, followed by colorectal, stomach, liver, and breast cancers [49].

A challenge among cancer therapy is replacing conventional treatments, such as chemotherapy or radiotherapy, with different alternatives, which could include the use of metallic nanoparticles, used as delivery media. The main reason for this need of replacement is determined by the side effects of conventional procedures, which may damage not only the tumor tissue but also healthy cells [50]. Currently, chemotherapy treatment aims to kill rapidly dividing cells, making no distinction between healthy cells and cancer cells. Using chemotherapy as means of treatment results in the destruction of cells that are fast proliferating (i.e., hair follicles and intestinal epithelium cells) [51]. Moreover the current medication (e.g., doxorubicin, daunorubicin, bleomycin, and cisplatin) used in cancer therapy is considered to be not fully effective, followed by other disadvantages, such as restriction by lack of specificity, high cost, high toxicity, and resistance susceptibility [5, 52].

Nanoparticles proposed to be used toward cancer therapy have been developed in a wide variety of shapes and a broad range of sizes, with the purpose to reduce the release rate and amount of required drug dose [53]. Not only nanometric materials such as dendrimers, micelles, and liposomes but also polymeric, ceramic, and metallic nanoparticles are currently of interest [54, 55]. Currently, different types of nanoparticle systems are investigated, with a particular emphasis on metallic nanoparticles, mostly due to their significant medical potential developed by allowing themselves to be conjugated with antibodies, ligands, and drugs [56]. Gold, silver, and platinum are some of the most researched materials to be used as nanometric particles in different areas of medicine [57].

Silver nanoparticles have been found to induce cytotoxicity via apoptosis and necrosis toward a range of different cell types. Moreover, they exhibit results against secondary effects of current therapies, as well, such as deoxyribonucleic acid (DNA) damage, generation of reactive oxygen species (ROS), increasing leakage of lactate dehydrogenase (LDH), and inhibiting stem cell differentiation [58, 59]. [Table 15.1](#)

Table 15.1 Silver nanoparticles used in cancer therapy.

Synthesis method of nanoparticles	Type of nanoparticles	Type of cancer cells	Therapeutic agent	Therapy	Ref.
Green synthesis using bacteria <i>Bacillus clausii</i>	<ul style="list-style-type: none"> - Spherical shape - 8–20 nm 	Human ovarian cancer A2780 cells	AgNPs + Salinomycin	<ul style="list-style-type: none"> - Higher level of ROS - DNA fragmentation - Enhanced LDH release - Alteration of cell morphology - Mitochondrial dysfunction - Generation of ROS - DNA fragmentation - Increased leakage of LDH - Loss of cellular viability - Cell viability and proliferation 	[59]
Green synthesis using molecule <i>resveratrol</i>	<ul style="list-style-type: none"> - Spherical shape - 3–20 nm 	Human ovarian cancer A2780 cells	AgNPs + Gemcitabine	<ul style="list-style-type: none"> - Increased level of ROS - Inhibited cell viability - Proliferation of HeLa cells - Change of mitochondrial membrane permeability 	[60]
Green synthesis using <i>sinigrin</i>	<ul style="list-style-type: none"> - Spherical shape - 20–40 nm 	Human cervical cancer HeLa cells	AgNPs + Camptothecin	<ul style="list-style-type: none"> - Increased level of ROS - Inhibited cell viability - Proliferation of HeLa cells - Change of mitochondrial membrane permeability 	[61]
Green synthesis using leaf extract of <i>Sesbania grandiflora</i>	<ul style="list-style-type: none"> - Spherical shape - 22 nm 	Human breast cancer MCF-7 cells	AgNPs	<ul style="list-style-type: none"> - Generation of ROS - Loss of cell membrane integrity - Promote apoptosis of cancer MCF-7 cells 	[52]
Green synthesis using leaf extract of <i>Vitex negundo</i> L. <i>induce</i>	<ul style="list-style-type: none"> - Spherical shape - 5–47 nm 	Human colon cancer HCT15 cell line	AgNPs	<ul style="list-style-type: none"> - DNA fragmentation - Inhibited proliferation of human colon cancer cell line HCT15 - Increased apoptosis 	[62]

Continued

Table 15.1 Continued

Synthesis method of nanoparticles	Type of nanoparticles	Type of cancer cells	Therapeutic agent	Therapy	Ref.
Green synthesis starch-capped AgNPs	<ul style="list-style-type: none"> - Spherical and polydispersed - 20–100 nm 	Human <i>colon</i> cancer HCT116 cancer cells	AgNPs	<ul style="list-style-type: none"> - DNA fragmentation - Promote apoptosis - Reduce the interaction between p53 and NF-κB 	[63]
Synthesis by sonication, centrifugation, and filtration	<ul style="list-style-type: none"> - Spherical - 30–50 nm (AgNPs powder coated with 0.2% PVP) 	Human <i>lung</i> cancer A549 cell line	AgNPs	<ul style="list-style-type: none"> - Generation of ROS - DNA fragmentation - Induced genotoxicity - Mitochondrial dysfunction - Early apoptosis 	[64]
Chemical synthesis—reduction of silver nitrate using sodium borohydride	<ul style="list-style-type: none"> - Spherical shape - 6 and 20 nm 	<ul style="list-style-type: none"> - Normal human <i>lung</i> fibroblast cells—IMR-90 - Human <i>glioblastoma</i> cells—U251 	AgNPs	<ul style="list-style-type: none"> - Production of ROS - Damaged DNA - Induced genotoxicity - Reduced ATP content - Mitochondrial dysfunction - Inhibited proliferation 	[65]

describes different applications of silver nanoparticles against various cancer cells, both as elementary therapeutic agents or in combination with other known drug agents, including salinomycin, gemcitabine, and camptothecin.

Yu-Guo Yuan and collaborators have shown that the synergism between silver nanoparticles and gemcitabine generated a higher cytotoxicity and apoptosis in A2780 cells, compared with the use of the therapeutic agent without nanoparticles [60]. Moreover, it was demonstrated that AgNPs can improve the responsiveness to gemcitabine or salinomycin in ovarian cancer cells, leading to an increased level of different proapoptotic genes, such as tumor protein p53, p21, Bax, and Bak, and activation of caspases 3 and 9. Furthermore a decrease in the levels of antiapoptotic B-cell lymphoma 2 genes was obtained in A2780 human ovarian cancer cells using AgNPs [59, 60]. In terms of colon cancers, an upregulation of p53, p21, and caspases 3, 8, and 9 along with a downregulation of AKT and NF- κ B has been observed following therapy with silver nanoparticles [63]. Fig. 15.2 represents the effect of wortmannin or AgNPs alone or the combination effect of wortmannin and AgNPs on apoptosis in cancer cells, as referenced in [66]. Apoptosis of cancer cells after treatment was assessed by the TUNEL assay; the nuclei were counterstained with DAPI. Representative images show apoptotic (fragmented) DNA (red staining) and the corresponding cell nuclei (blue staining). According to tumor volume and weight ratio of tumor tissue data, the

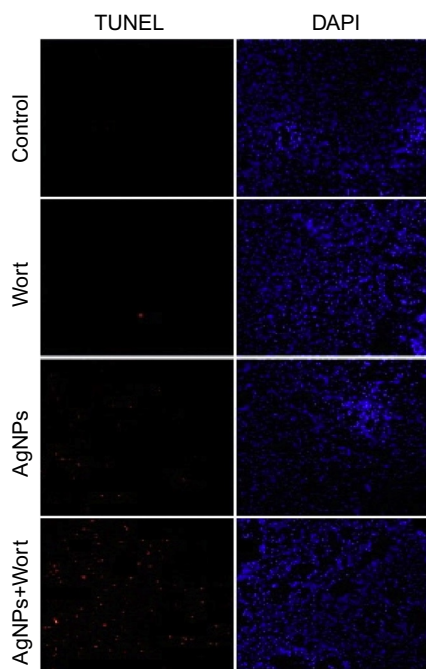


Fig. 15.2 The synergistic effect between silver nanoparticles and wortmannin against cancer cells in mice with melanoma [66].

terminal deoxyribonucleotidyl transferase (TDT)-mediated dUTP-digoxigenin nick-end labeling (TUNEL) assay revealed many more apoptotic cells in the tumor treated with AgNPs plus wortmannin than in the tumor treated with AgNPs alone, while few of the apoptotic cells were observed in the untreated tumor or the tumor treated with wortmannin alone.

The synergistic effect between silver nanoparticles and camptothecin in human cervical cancer cells (HeLa) was inferred from their ability to activate caspases 9, 6, and 3. Moreover, increased levels of p53, p21, cyt C, Bid, Bax, and Bak and modulated expressions of Akt1, RAF, MEK, Erk1/2, JNK, P38, NF- κ B, and Cyclin D, which are known as molecules involved in cell survival, were observed in HeLa cells after using AgNPs combined with camptothecin [61].

The effects of PVP-coated AgNPs and silver ions (Ag^+) against the human lung cancer were also reported. Even though a similar toxic effect on mitochondrial function of alveolar cell line, A549, was observed using both AgNPs and Ag^+ , it should be highlighted that the AgNPs lead to higher levels of ROS than Ag^+ (leading further to DNA damage) [64]. In addition to increased cytotoxicity, AgNPs not only inhibited cancer cell proliferation through induction of apoptosis but also inhibited the cancer cell migration [56, 67]. Regarding the toxicity effects of starch-coated silver nanoparticles, it was reported that their use could lead to the ATP depletion, mitochondrial damage, and cell cycle arrest in G2/M phase, in relation to human lung fibroblast cells and human glioblastoma cells [57].

Furthermore, it was shown that hybrid nanoparticles, composed of silver and bioactive small molecules of quinacrine based on poly(lactic-*co*-glycolic acid) (PLGA), exhibit antitumor potential in H-357 oral cancer cells and oral squamous cell carcinoma-cancer stem cell (OSSC) [62]. The presence of cancer stem cells has been found to be the main cause of cancer relapse even after successful surgeries took place and also for the development of cancer resistance to treatment [68]. Therin, Satapathy, and coworkers have demonstrated that their obtained hybrid nanoparticles were able to generate apoptosis in OSCC inducing S-phase arrest and damaging the DNA [62].

15.2.2 AgNPs as antiviral agents

The development of resistance to treatment in various pathogens including viruses represents another major cause of death, which is a major concern of the medical, pharmaceutical, and biotechnological systems [18]. Silver nanoparticles are employed in newly emerging applications as antiviral agents, due to their inhibitory activity against numerous viruses, including certain strains of coronavirus [68a], hepatitis, influenza, herpes, recombinant respiratory syncytial virus, and human immunodeficiency virus [47]. It is generally acknowledged that silver nanoparticles contribute to viral inactivation because of reactions with sulfhydra, amino, carboxyl, phosphate, and imidazole groups [69].

In 2017 worldwide about 1 million people died only from AIDS-related illnesses [69]. Even if over 25 antiretroviral compounds have been developed and accepted for use in HIV-infected individuals, they do not generally have the capacity to completely eradicate viral reservoirs. This phenomenon often occurs as a result of the

virus developing resistance to the treatment [70]. AIDS can be caused by two different lentiviruses, HIV-1 and HIV-2. Research carried out in various countries indicates that up to 78% of patients infected with HIV-1 and treated by antiretroviral administration have developed resistance to at least one of the available drugs [71]. Considering these developments, nanotechnology challenges have emerged in relation to the eradication of latent HIV reservoirs [72]. There are three main pathways, in connection with virus infections, which are involved in limiting of pathogen replication in infected cells, namely, apoptosis, necroptosis, and pyroptosis [73]. While apoptosis is usually nonimmunogenic, it has a vital role in eliminating virus-infected cells. For viruses that successfully resist apoptosis, programmed necrosis and pyroptosis may be vital to eliminate the viral factor before adaptive immunity is engaged [74]. Elechiguerra and coworkers demonstrated that silver nanoparticles exhibited antiviral activity performed through gp120 glycoprotein knobs, which have the main function based on binding to the CD4 inhibiting fusion or entry of the virus into the host cell receptor sites [75]. It has been argued that acting via gp120 represents an optimal manner by which CD4-dependent virion binding, fusion, and infectivity of HIV can be prevented [71]. Not only proper functioning of silver nanoparticles occurred at an incipient stage of virus replication, but also a very important point is that they have developed inhibition of HIV-1 life cycle at postentry stages [71, 75]. Silver nanoparticles, synthesized using HEPES buffer and human serum albumin, which served as a stabilizer, have been reported to exhibit cytoprotective and postinfected anti-HIV-1 potential toward Hut/CCR5 cells. In that study the results have shown that the apoptosis of the cells is dose dependent. For example, a significant reduction of apoptotic cells, from 49% to 19%, was obtained for a higher concentration of nanoparticles, (50mM), while a concentration of 5 mM leads to cell apoptosis up to 35%. Moreover, it was confirmed that silver nanoparticles exhibit cytotoxicity activity, in particular against HIV-1 cells, the survival percentage of 80% for the Hut/CCR5 host being registered at concentration up to 50-mM nanoparticles [76]. PVP-coated AgNPs have also been used in studies against HIV, besides their potential in treating cancer, using an *in vitro* human cervical tissue-based organ. The PVP-coated Ag nanoparticles have been found to inhibit HIV-1 transmission within a relatively short period of time, in about 1 min. It has been observed that this delivery drug system protects the cervical tissue against infection with HIV-1, for 48h, even after several successive washes, providing long-term protection of cervical tissue from infection [71]. Moreover, after the inactivation of HIV-1 and blocking of viral entry, the fusion of HL2/3 and HeLa CD4 cells was also blocked, in a dose-dependent manner [71]. Likewise, PVP-coated AgNPs (0.2-wt% PVP—pharmacologically inactive substance) were shown to inhibit the herpes simplex virus type 2 cytopathic effect on host cells, causing a significant reduction of progeny viruses. A concentration of 100- μ g/mL AgNPs induced an insignificant toxicity on Vero host cells being as well capable of inhibiting HSV-2 replication. A different delivery drug system based on silver nanoparticles capped with mercaptoethane sulfonate, sonochemically synthesized, was used for the inhibition of herpes simplex virus type 1, having no cytotoxic effect on host cells. Because the interaction between viral envelope glycoproteins and cell surface heparan sulfate affects the attachment and entry of the virus into cells, silver nanoparticles should lead to the

blockage of viral entry into the cell and to the prevention of subsequent infection [77]. In vivo studies showed that tannic acid–modified silver nanoparticles (Ta-AgNPs) exhibit significant antiviral activity against herpes simplex type 2 infection. By treating intravaginally a mouse model with HSV-2 infection with Ta-AgNPs, an increase in the percentage of IFN-gamma + CD8 + T-cells, activated B cells, and plasma cells at the tissue level was observed [78].

Another important application of silver nanoparticles developed for antiviral purposes concerns the treatment of influenza. A significant number of results were reported concerning the inhibitory activity of this kind of nanoparticles against the H1N1 virus, followed by the H3N2 virus. A delivery system for zanamivir medication, used to treat and prevent influenza, was shaped at nanoscale alongside silver nanoparticles through a simple chemical method using a solution of vitamin C. Using a similar method a surface enrichment of the AgNPs with amantadine was developed, for the purpose of overcoming drug resistance shown by the H1N1 virus [79]. It was reported that zanamivir and amantadine self-assembled on the surface of AgNPs to get better inhibitory potential of neuraminidase and hemagglutinin activity, compared with single drug treatments. Furthermore, structural changes in MDCK host cells caused by H1N1 viral invasion were considerably diminished by inducing DNA fragmentation, chromatin condensation, and activation of capase-3 using the codelivery of the zanamivir and amantadine nanosystems, coupled with AgNPs [80]. Per this report the DNA fragmentation and nuclear condensation depressed by zanamivir-activated silver nanoparticles were shown in Fig. 15.3. The MDCK cells were treated with or without zanamivir-activated silver nanoparticles after H1N1 virus infection, followed by detection with TUNEL-DAPI costaining assay.

Xiang and collaborators have reported that AgNPs hold positive outcome in preventing H3N2 influenza virus infection, both in vitro and in vivo. In vitro studies

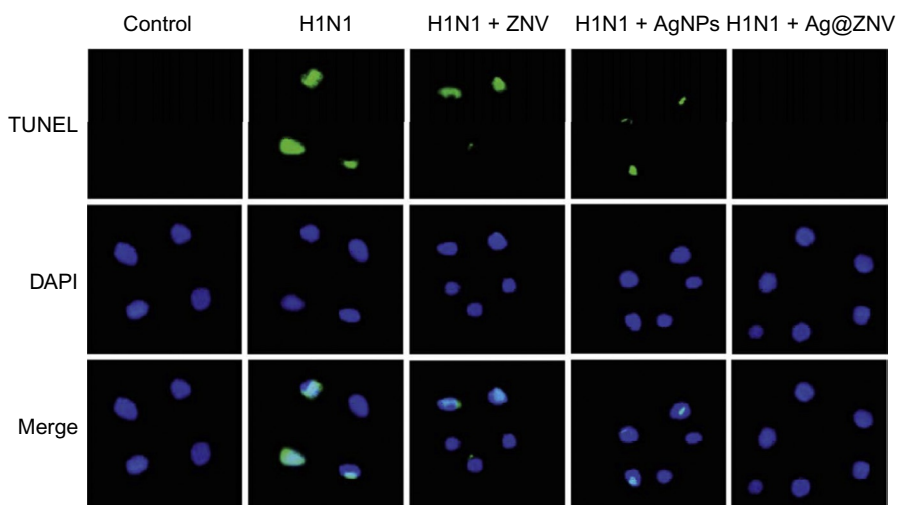


Fig. 15.3 The synergistic effect between silver nanoparticles and zanamivir against H1N1-infected MDCK cells [80].

confirmed that the growth of the virus hemagglutinin activity is inhibited in a dose-dependent manner of AgNPs. It also has to be taken into consideration that a too high concentration of AgNPs can have a toxic effect on MDCK cells. Reducing the concentration of silver nanoparticles, from 100 to 50 $\mu\text{g}/\text{mL}$, caused a significant decrease of the cytotoxicity toward MDCK cells. Furthermore, silver nanoparticles protect the cells against viral infection, decreasing the cellular apoptosis induced by the H3N2 influenza virus. It was shown on an influenza-infected mouse model that AgNPs, administered via intranasal absorption, improved the survival rate in mice, by preventing the growth of virus in their lungs and inhibiting the development of pathologic lung lesions [81].

The hepatitis viruses are among the most common viruses that can cause persistent infections that may lead to cancer [82]. In this sense, it is desired to increase the access to curative therapies of hepatitis virus infections. Consequently the effects of silver nanoparticles were explored on hepatitis viruses as well. Monodisperse silver nanoparticles having dimensions between 10 and 50 nm in diameter were able to reduce the extracellular hepatitis B virus DNA formation of HepAD38 human hepatoma cell line and could inhibit in vitro production of HBV RNA and extracellular virions [83] (Table 15.2).

15.2.3 AgNPs as antimicrobial agents

Increased use of antibacterial agents has resulted in bacteria developing resistance to antibiotics. Consequently the development of alternative treatment paths is of paramount importance. Silver has been used during the years for a variety of medical purposes [86]. The antimicrobial potential of silver is well known, and it has been the main research subject for the use of silver and more specifically of silver nanoparticles, which exhibit increased biochemical activity due to their large surface-to-volume ratio and surface characteristics (structure, roughness, etc.).

Dakal and coworkers remarked that silver nanoparticles can be engineered so as to increase their efficacy, stability, specificity, biosafety, and biocompatibility [87]. The mechanisms by which silver nanoparticles are thought to work against pathogens are different, many of which are based on adhesion to microbial cells, penetration inside the cells, ROS and free radical generation, and modulation of microbial signal transduction pathways [88]. In view of their dimensions, silver nanoparticles can penetrate the cells and inhibit enzymatic systems in the respiratory chain of bacteria, thus affecting their DNA synthesis [89].

The antibacterial activity of silver nanoparticles has been investigated individually, as well as in conjunction with antibiotics, thus potentially leading to a synergistic effect, usually against microorganisms that have developed resistance to common antibiotics. *Staphylococcus aureus* is one of the most important pathogens that cause a wide range of clinical infections [90]. The occurrence of *Staphylococcus aureus* bacteremia and its complications were frequently observed in the last years because of the increased number of cases of invasive procedures, immunocompromised patients, and resistance of certain *Staphylococcus aureus* strains to available antibiotics [91].

Table 15.2 Silver nanoparticles used in antiviral therapy.

Synthesis method of nanoparticles	Characteristics	Virus and host cells	Therapeutic agent	Activity	Ref
Chemical synthesis using HEPES buffer	5–20 nm	HIV-1 in Hut/CCR5 cells	AgNPs	<ul style="list-style-type: none"> – Potent cytoprotective – Inhibition of viral replication in Hut/CCR5 cells – Reduction of HIV-associated apoptosis 	[76]
PVP-coated AgNPs homogenized in nonspERMICIDAL Replens gel	<ul style="list-style-type: none"> – 30–50 nm – Spherical 	HIV-1 in human cervical tissue	AgNPs + PVP	<ul style="list-style-type: none"> – Provides HIV-1 virucidal activity – Blocks the infection of cell-free and cell-associated HIV-1 	[71]
Chemical synthesis-oxidation reduction	<ul style="list-style-type: none"> – Spherical – 9.5 nm 	H3N2 in MDCK cells	AgNPs	<ul style="list-style-type: none"> – Reduction of H3N2-associated apoptosis – Destruction of morphologic viral structures – Decreases in hemagglutinin titer 	[81]
Chemical synthesis using HEPES buffer	5–20 nm	H1N1 in MDCK cells	AgNPs	<ul style="list-style-type: none"> – Inhibition of activity on H1N1 influenza A virus – Reduction of H1N1 influenza A virus-associated apoptosis toward MDCK cells 	[84]
Synthesis using vitamin C, ultrasonicated	2–2.3 nm	H1N1 in MDCK cells	AgNPs + zanamivir	<ul style="list-style-type: none"> – Decreased the neuraminidase activity of H1N1 influenza virus – Reduction of H1N1 virus-associated apoptosis – Mediated apoptosis via ROS generation 	[80]

Chemical synthesis using HEPES buffer	10–50 nm	HBV	AgNPs	<ul style="list-style-type: none"> – Inhibition of hepatitis B virus replication – Inhibition of the in vitro production of HBV RNA and extracellular virions 	[85]
Chemical reduction using sodium citrate	33 nm	HSV-2 strain 333 Vero cells	AgNPs + tannic acid	<ul style="list-style-type: none"> – Mount early immune response – Helps to boost antiviral immunity 	[78]
Sonochemical synthesis	<ul style="list-style-type: none"> – Spherical – 4 nm 	HSV-1	AgNPs + mercaptoethane sulfonate	<ul style="list-style-type: none"> – Ag-MES nanoparticles did not affect the mitochondrial activity – Inhibition of HSV-1 infectivity 	[77]

Generally, silver nanoparticles that have been obtained by various so-called “green” synthesis procedures are preferred, because the final products would be more suitable when applying them in the medical and pharmaceutical area. Kaviya et al. reported the biosynthesis of silver nanoparticles using *Citrus sinensis* peel extract, which were further tested for their antimicrobial potential against *Staphylococcus aureus*, with good results [92]. Antibacterial activity against multidrug resistant *Staphylococcus aureus* was also observed due to silver nanoparticles micosynthesized extracellularly using *F. acuminatum* Ell. and Ev. isolated from ginger (*Zingiber officinale*) [93]. Furthermore, silver nanoparticle-impregnated bacterial cellulose (produced by *Acetobacter xylinum*) was found to possess antimicrobial activity against *Staphylococcus aureus* bacteria [94]. Mirzajani’s study [95] confirmed that part of antibacterial concern is involved with wall damage and accumulation of AgNPs in the bacterial membrane. Moreover, it has been found that a rate of change in the α -helix position of the peptide chain and glycan strands may also consequently be affected by the presence of AgNPs [95]. Moreover the antibacterial potential of known antibiotics (such as penicillin G, kanamycin, amoxicillin, erythromycin, clindamycin, chloramphenicol, ampicillin, and vancomycin) was increased in the presence of AgNPs against *Staphylococcus aureus* [96]. The hypothesis of Kim’s group that silver nanoparticles can also be used for antimicrobial control systems was further tested in the Abbaspour’s study, which reported a sensitive and highly selective dual aptamer-based sandwich immunosensor for the detection of *Staphylococcus aureus* [97, 98].

Streptococcus pyogenes and *Streptococcus pneumonia* are other major pathogens that are responsible for a wide range of diseases such as pharyngitis, erysipelas, septicemia, meningitis, pneumonia necrotizing fasciitis, and streptococcal toxic shock syndrome [99]. In a study demonstrating the bactericidal effect against erythromycin-resistant *Streptococcus pyogenes*, it was shown that silver nanoparticles work by inhibiting cell wall synthesis, protein synthesis mediated by the 30s ribosomal subunit, and nucleic acid synthesis [100]. Besides the fact that silver nanoparticles possess antimicrobial potential against *Staphylococcus aureus*, it was shown that this bacterium has the ability to reduce extracellularly silver ions in silver nanoparticles. Nanda and collaborators have used *Staphylococcus aureus*-mediated AgNPs against human pathogenic microorganisms, using both gram-positive and gram-negative bacteria. The first category, namely, gram-positive bacteria, including *Streptococcus pyogenes*, was more susceptible to the mentioned antimicrobial agent [101]. *Escherichia coli* was used as a model for gram-negative bacteria exposed to the antimicrobial activity of silver nanoparticles. The results showed that the assayed bacteria were damaged at the cell wall level, as a result of the accumulation of silver nanoparticles in the bacterial membrane [102]. The bactericidal effect of silver nanoparticles (biosynthesized using *Salmonella typhimurium* as the reducing agent) was further observed against *E. coli*, after disc diffusion tests [103]. Furthermore the synergism of silver nanoparticles with amoxicillin and polymyxin B, respectively, was investigated against *E. coli*. The results showed that the delivery systems led to greater bactericidal efficiency, compared with the situation when the therapeutic agents are administrated individually [104, 105].

P. aeruginosa is a notable nosocomial pathogen, which mainly affects patients with neutropenia and those who are immunocompromised [106]. Salomoni and coworkers

evaluated in their study the antimicrobial potential of different concentrations of commercial 10-nm AgNPs on two acquired nosocomial infectious strains of *P. aeruginosa*, resistant to a significant number of antibiotics. Various profiles of susceptibility to antibiotics and AgNPs were observed [93]. The essential mechanism concerning the effect of silver nanoparticles against multidrug-resistant *P. aeruginosa* consists in the disequilibrium of oxidation and antioxidation processes and the failure to eliminate the excessive ROS [107]. Moreover, it was observed that *P. aeruginosa*, which can extracellularly biosynthesize thermodynamically stable and the desired size and shape of silver nanoparticles, exhibited susceptibility toward the same silver nanoparticles that it contributed to their synthesis [108]. These results recommend the further development of silver nanoparticles, as a consequence of the multidrug resistance phenomena [109].

The replication of the results presented herein was verified by the authors, with comparable results. To synthesize silver nanoparticles, 1 μL of bacterial strains (*E. coli* and *P. aeruginosa*, respectively) were freshly inoculated into conical flasks containing 100 mL of liquid medium (0.6-g/L yeast extract, 1-g/L sodium nitrate, and 3-g/L glucose) at 33°C for 48 h. After 48 h the cultures were centrifuged at 4000 rpm for 30 min using an angular rotor centrifuge. Ten milliliter of supernatant was mixed with 90 mL of precursor (1-mM aqueous AgNO_3 solution). The precursor was first autoclaved (sterilized) at 128°C for 30 min. The samples were incubated for 48 h at 33°C and 150 rpm (to ensure maximum enzymatic activity of the extract). For the purification of the biosynthesized AgNPs, the samples were centrifuged at 4000 rpm for 30 min. The collected pellets were washed with 25 mL of distilled water and dried at 80°C until the liquid was evaporated. This last step was performed four times, followed by sample drying in an oven at 200°C for 6 h. The potential of silver nanoparticles for antimicrobial activity was determined using the disk diffusion method (as described in the Clinical and Laboratory Standards Institute (CLSI): M02-A11 standard (Performance standards for antimicrobial disk susceptibility testing)), by impregnation of 15 μL of solution containing AgNP on each 6-mm disc containing also 5- μg ciprofloxacin, thus investigating the potentially synergistic effect of silver nanoparticles in combination with the antibiotic. Discs containing ciprofloxacin 5 μg , without the addition of AgNPs, were used for control. The discs were placed on the surface of the bacterial culture (*E. coli* and *P. aeruginosa*, respectively). After 24 h of incubation at 31°C, the inhibition diameter was measured and compared with the control samples. The results are presented in Fig. 15.4, where the inhibition diameter variation can be seen, as function of the precursor concentration, and type of bacteria used for the synthesis of the silver nanoparticles. The synergistic effect between the antibiotic and the silver nanoparticles is evident, even more so against *E. coli*, compared with the effect against *P. aeruginosa*. Moreover the antibacterial effect is significantly increased for higher concentrations of the precursory solution.

The fungistatic and fungicidal effects of silver nanoparticles against *Candida* species were found to increase with their stabilization using polymers and surface-active agents. Furthermore the antimicrobial potential of silver particles at nanoscale is comparable with the potential of ionic silver, which is cytotoxic at required concentrations [110]. Silver nanoparticles induce apoptotic cell death in *Candida albicans* through the increase of hydroxyl radicals and reactive oxygen species [111]. Positively charged

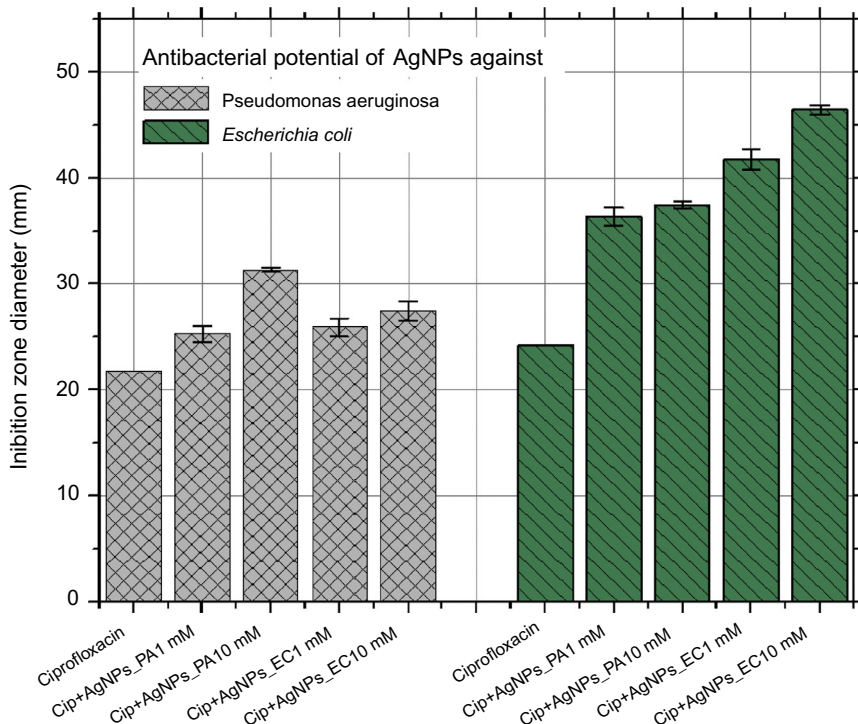


Fig. 15.4 The synergistic effect between silver nanoparticles and ciprofloxacin, against *Escherichia coli* and *Pseudomonas aeruginosa* cultures.

silver nanoparticles disrupt the cell membrane of *Candida albicans*, through the electrostatic attraction between the negatively charged cell membrane of the microorganism and the positively charged nanoparticles, permeabilizing the outer cell wall as it becomes rough and distended, allowing the nanoparticles to penetrate the cell, leading to an inhibition of the filamentation of the yeast [112, 113].

In the same manner an aqueous extract from the filamentous fungus *F. oxysporum* mediated the synthesis of silver nanoparticles, which showed further a high antifungal potential. Their potential antifungal activity was assessed against *Cryptococcus neoformans*, thus observing a disruption of the cell wall and loss of the cytoplasm content [114].

15.3 Concluding remarks and future outlook

The ability to synthesize controlled size and shape silver nanoparticles has greatly improved in the recent years, due to the efforts of the scientific community. However, there is always room for improvement, in terms of experiment replicability, and the transfer from the laboratory to the manufacturing and production side. The combination between silver nanoparticles and other compounds (natural or synthetic antibiotic

and anticancer or antiviral compounds) leads to a synergistic effect, which could potentially help to develop new treatment strategies, thus overcoming the current limitations in terms of microbial resistance, more and more encountered in the recent years. The next logical step would be to test these compound/silver nanoparticle combinations using *in vivo* conditions, to a greater extent. These types of experimental investigations may clarify some pharmacological mechanisms, involving the delivery, release, and interaction of the delivery system with the human body. In the future, human testing will be a necessary step that will assess the appearance of side effects, if any, and will be a strong point in determining whether the development of these types of treatments that include nanoparticles is a viable path to be pursued. Regardless of the benefits mentioned herein, some reports are already suggesting that antimicrobial resistance to the treatment starts to develop, even when silver nanoparticles are included [115, 116]. Consequently the development of a clinically applicable treatment protocol using silver nanoparticles is a daunting task, even if a significant number of positive reports can be found in the literature.

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