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Perspective

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The medical uses of silver: history, myths and scientific evidence

Serenella Medici,[a*] Massimiliano Peana,[a*] Valeria M. Nurchi,[b] Maria Antonietta Zoroddu[a]

[a] Department of Chemistry and Pharmacy, University of Sassari, 07100 Sassari, ITALY

[b] Department of Chemical and Geological Sciences, University of Cagliari, 09042 Cagliari, ITALY

Abstract

Silver has no biological role and it is particularly toxic to lower organisms. Although several silver formulations employed in medicine in the past century are prescribed and sold to treat certain medical conditions, most of the compounds, including those showing outstanding properties as antimicrobial or anticancer agents, are still in early stages of assessment, that is, *in vitro* studies, and may not make it to clinical trials. Unlike other heavy metals, there is no evidence that silver is a cumulative poison, but its levels can build up in the body tissues after prolonged exposure leading to undesired effects. In this review, we deal with the journey of silver in medicine going from the alternative or Do-It-Yourself drug to scientific evidences related to its uses. The many controversies push scientists to move towards a more comprehensive understanding of the mechanisms involved.

1 Introduction

Sometimes the impulse to write a review may arise from a casual conversation with a layperson under unusual circumstances. In this case, the man in the street approached the Chemistry bench during a Night of the Research event and proudly stated he was doing his own chemistry at home, preparing colloidal silver for everyday uses with a recipe found on the internet. Puzzled and curious, the Chemist decided to check this out and, while surfing the net, came across a video showing a "blue man" drinking a glass of silver solution. The Chemist, even more intrigued by the topic, jumped from one site to the other, from tutorials to all kinds of do-it-yourself videos and blogs, and found there are

thousands, maybe millions, people in the world taking or even making their own "colloidal", "ionic", "ionic colloidal" silver at home, unsupervised, using a 9 V battery, pure water (sometimes adding a bit of kitchen salt) and silver rods or coins, hoping to treat "more than 650 diseases, pathogens and other conditions" or "kill more than 650 different germs, viruses, bacteria, and fungi within 5-7 minutes". Completely fascinated, the Chemist searched the net to make a list of such diseases: arthritis, cancers, viruses (including herpes, influenza, HIV, Ebola, SARS and West Nile), bacterial and fungal infections (*Staphylococcus* sp., *Streptococcus* sp. – including *pneumoniae* –, *Neisseria meningiditis*, *Salmonella* sp., *Candida albicans*), gastrointestinal conditions (ulcer, diarrhea, stomach bug and colitis), skin problems (acne, warts, dermatitis, eczema, psoriasis, seborrhea, hemorrhoids, lupus, rash), eye infections, appendicitis, cystitis, diphtheria, pyorrhea, poliomyelitis, scarlet fever, tetanus, syphilis, malaria, typhus and cough. Enough? No, because claims have been made that silver can also relieve allergies, diabetes, catarrh, chronic fatigue syndrome, and problems with the nervous and locomotor systems. According to the net, silver "is effective against bad bacteria, but leaves good ones (those living in our intestine) healthier than ever", and "it acts as a second immune system".

The Chemist, finally, connected to the scientific publication databases and began to work.

1.1 Biological role and fate

Silver is a transition metal belonging to the so-called "noble" group.[1-3] As many other metals,[4-9] it has no biological role and it is especially toxic to lower organisms. Food is a source of silver: flour contains up to 0.3 ppm, bran about 1 ppm, fish 10 ppm, milk 50 ppb, meat 40 ppb.[10] It can be said that in general the content of silver in foods lies in the 10-100 μg/kg range, while in natural waters it is 0.2-0.3 μg/l.[11] Human daily intake can be as high as 20-80 μg depending on the diet,[12,13] but only 10% of the ingested metal is absorbed, the rest being excreted via the intestine, mainly via desquamation of silver containing cells of the gastrointestinal system. This has been determined after radiosilver (^{110m}Ag) administration to different mammals (monkeys, dogs, mice and rats) by oral, intravenous or intraperitoneal routes: over 90% of this isotope was eliminated in the feces, meaning

that only 10% or less of the oral dose was absorbed. The study evidenced that the whole body retention in monkeys, rats and mice was rather low, less than 1% of the initial dose after one week, while in dogs the amount was higher, although lower than 10% of the initial dose.[14] The reticulo-endothelial organs seem to be responsible for the highest retention of this metal. After intravenous administration, silver was found in spleen, liver, bone marrow, lungs, muscle and skin tissues, with concentrations decreasing in this order.[15] Other studies evidenced that silver salts can be taken in through the gastrointestinal system, the lungs, and the epithelia of skin, conjunctiva, and nasal mucosa. Once inside the body, silver can accumulate and be stored in the reticulo-endothelial cells of spleen, liver, mucous membranes and skin, in basement membranes (e.g. renal glomerulus), possibly in bone marrow, and probably in muscles.[16-18] It may also cross the blood-brain barrier and accumulate in neurons and glia.[19,20]

The concentration of silver in blood, measured by spectroscopic techniques, was found to be rather elevated in people exposed to this metal for occupational reasons (workers in bullion manufacture, tableware production, chemical industry, jewelry making and silver reclamation), ranging from 0.1 to 23 μg/l, while blood silver levels in unexposed subjects normally range from 0.1 to 0.2 μg/l.[21]

Metallic silver under the form of nanoparticles can enter the body mainly via ingestion or inhalation.[22-24] Macrophages are the first cells that they encounter once inside. Silver nanoparticles can be incorporated (Fig. 1) via a mechanism which takes different paths depending on the type of cell: pinocytosis, endocytosis dependent on caveolae and lipid raft composition, clathrin-dependent endocytosis and phagocytosis.[25] Uptake kinetics, intracellular localization and exocytosis also depend on nanoparticle size and surface characteristics, as well as on the ability to form aggregates. After internalization, these nanoparticles can reach the intracellular targets, such as the endoplasmic reticulum, mitochondria, cytoskeleton and nucleus, and interact with them in different ways.[26,27]

<FIGURE 1>

Unlike other heavy metals, such as mercury, cadmium or lead, there is no evidence that silver is a cumulative poison,[28] but its concentration can build up in the body tissues after prolonged exposure leading to undesired effects (*vide infra*). Information about the biotransformation of silver cations once they are absorbed by the organism, except their reduction and deposition as metallic silver in the tissues, is poor or lacking. So what are we able to infer about the effects of silver inside the body from the chemical data?

1.2 Speciation in biological environments

Silver as a metal is insoluble in water, while it is soluble in its cationic form. Metallic silver and most its inorganic compounds can ionize and release biologically active Ag^+ in the presence of water and an oxidant, a condition that can be found in body fluids and other secretions. Solubility of silver salts varies according to the nature of the anion. Silver nitrate $AgNO_3$ has a very high solubility (122 g / 100 ml of water), silver acetate CH_3COOAg rather low (1.02 g /100 ml of water, $K_{sp} = 1.94x10^{-3}$) while silver chloride AgCl is nearly insoluble ($K_{sp} = 1.8x10^{-10}$), similarly to silver hydroxide AgOH ($K_{sp} = 1.52x10^{-8}$), but the latter is unstable and tends to form insoluble silver oxide Ag_2O over time. Silver phosphate Ag_3PO_4 ($K_{sp} = 2.7x10^{-18}$) is less soluble than the chloride, while silver sulfide Ag_2S ($K_{sp} = 8.0x10^{-51}$) and selenide Ag_2Se ($K_{sp} = 1.0x10^{-53}$-$1.0x10^{-59}$) are amongst the most insoluble silver compounds, and thus the most unreactive and less associated with toxic effects.

In a hypothetical massive ingestion of ionic silver (supposing death would not come first), most of it would be converted into insoluble AgCl by the hydrochloric acid in the stomach (0.1 M, approximately) and the KCl and NaCl also present in the gastric fluids. The solubility of Ag^+ should drop further in the presence of high concentrations of chloride ions, as are present in the stomach, due to the common ion effect, but this situation also favors the formation of the $AgCl_2^-$ complex,[29,30] which instead is soluble. The tendency of silver-sulfur compounds to bind with other sulfur-containing gastric ligands from food or within the mucosal lining could account for their poor solubility.

The fate of ingested metallic silver, especially in its nanometric form, can be slightly different in the fact that the hydrochloric acid in the stomach can (partially) dissolve it to form ionic silver,[31] which undergoes all the previously mentioned transformations, while the undissolved metal proceeds further to the intestine and the bloodstream, to be translocated throughout the human body[32] or excreted. This was demonstrated by a recent study[33] that investigated the gastrointestinal fate of both silver nanoparticles and silver ions from a commercial dietary supplement by preparing a model of the human gastrointestinal tract. The study showed that the neutral pH of the mouth and the presence of biomoloecules in the saliva (able to form a corona), prevented the dissolution or the aggregation of the nanoparticles, while the same biomolecules formed complexes with the silver ions. On the other hand, the low pH and the presence of chloride ions in the stomach caused extensive dissolution of the nanoparticles in this organ, and no aggregation. It was also possible to quantify the amount of dissolved silver: in the fed condition (i.e. after meal), 72% nanoparticles (by mass) dissolved, with 74% silver ions forming Ag-biomolecule complexes and 26% forming AgCl; in the fasted condition (i.e. before meal), 76% nanoparticles dissolved, with 82% silver ions forming Ag-biomolecule complexes and 18% forming AgCl. The environment in the small intestine prevented further dissolution or aggregation of silver nanoparticles, while the silver ions formed only Ag-biomolecule complexes.

Thus, silver ions, as seen, have a strong affinity for proteins, especially albumins, metallothioneins, and macroglobulins, but also for reduced glutathione, hence free Ag^+ ions can form stable complexes with these species not only in the gastrointestinal tract, but also in the blood or inside the cells. Finally, they can meet sulfides and/or selenides and precipitate as insoluble dark compounds.

The Standard Reduction Potential (SRP) for the couple Ag^+/Ag is 0.7994 V at pH below 6, but at higher pH it is slightly lower since Ag_2O starts forming, therefore the couple Ag_2O/Ag must also be considered (0.34 V at basic pH). This means that a variety of both oxidants and reductants present in the biological fluids are able to play a role in the oxidation state(s) of silver inside the body or in other biological media. The SRP for AgCl is 0.222 V in slightly acidic solutions, like those found inside

the vacuoles and lysosomes. There, metallic silver can also react, for instance, with hydrogen peroxide potentially present, forming silver ions:[25]

$$2Ag + H_2O_2 + 2H^+ \rightarrow 2Ag^+ + 2H_2O \ (E_0 = 0.17 \text{ V})$$

On the other hand, sunlight photons can trigger Ag^+ reduction, in a reaction similar to that employed in black-and-white photography development, so silver inside the body can be deposited as metal nanoparticles under the skin, especially in sun-exposed areas.

1.3 Lethal doses and toxicity

The evaluation of a metal's toxicity is strictly correlated to its bioavailability, and determined by its solubility, oxidation state, complexation ability towards biological targets (i.e. proteins or other coordinating species), excretion and detoxification routes. Lethal silver doses have been evaluated in both its soluble and insoluble forms for a number of animal species, including mammals (rats and dogs), but not for humans, for whom they can only be inferred. The negative effects of silver ions and nanoparticles on human health, however, are constantly being evaluated and reported.[34] As a matter of fact, soluble compounds are always more toxic than insoluble ones.

The estimate acute lethal dose of $AgNO_3$ for humans seems to be at least 10 g,[35,36] while other sources report that the estimated LD_{50} is considered to be 28 mg/kg.[37] The systemic effects of a lethal dose are anticipated by severe hemorrhagic gastroenteritis and shock. Goodman and Gilman reported that silver ions seem to first stimulate and then depress structures in the brain stem. A rise in blood pressure is then caused by central vasomotor stimulation. Concurrently bradycardia develops after central vagal stimulation. Finally death occurs, due to respiratory depression.[38]

Why is silver nitrate so toxic? In general, Ag(I) binding to proteins causes their denaturation, which is thought to be the reason behind $AgNO_3$'s corrosive and caustic effects on inner mucosae. Moreover, this silver salt is also a strong oxidant, able to produce superoxide radicals and hydrogen peroxide,[39]

and to extensively oxidize biochemical molecules. On the other hand, silver ions *per se* seem to be related with a decreased proliferative capacity, loss in cellular identity, and degenerative modifications in the nucleus and cytoplasmic organelles.[40] Dissimilarly, nitrates act as vasodilators, which may lead to hypotension and circulatory collapse, and are able to interact with oxyhaemoglobin, leading to the formation of methaemoglobin which can rapidly lead to cyanosis and death due to hypoxia.[41,42]

Silver nitrate ingestion is not very common, and there are just a few cases reported, both as intentional (suicide attempts) or involuntary (misunderstood prescriptions) ingestions. The outcome of $AgNO_3$ poisoning depends on a timely medical treatment. Moderate to severe damage (burns, erosion and striction) to the oral, esophageal and gastric mucosa have been observed due to the caustic properties of this salt, but also vomiting, respiratory distress syndrome and collapse of the lungs. In rare cases, death has been reported.[43]

Silver acetate has an LD_{50} of 36.7 mg/kg in mice, reflecting its relative solubility. Lower doses of this salt caused ataxia in mice, together with hyper-excitability, labored breathing, central nervous system depression, and even death.[44] Since silver acetate has been used to treat nicotine dependence,[45,46] due to its probable toxicity the U.S. FDA recommended that its intake be limited to about 750 mg over a short period of time.[46]

Silver chloride, instead, due to its poor solubility was found to have an LD_{50} higher than 10 g/kg in mice following oral administration, suggesting that, as expected, AgCl is much less toxic than $AgNO_3$. However, its lethal dose in humans still remains unknown.

Once silver ions accumulate in body tissues, especially the skin, they can be reduced to metal nanoparticles by sunlight-induced processes and other reductants, or be precipitated as insoluble dark sulfides and selenides, thus staining the cutis in different shades of grey-blue. It has been suggested that silver ions could also trigger melanin overproduction, increasing the dark hue of the skin.[47,48] Such discoloration, called argyria (or argyriosis when localized in the eyes), can be caused by long-term inhalation and/or ingestion of silver compounds, but it is considered as a merely cosmetic

condition because it does not seem to induce any toxic effects on the body. Nevertheless, the discoloration is permanent, meaning that it cannot be removed by chelation therapy, dermabrasion or laser erasing treatments.[48-50]

Argyria has been recognized and documented since the 19th century when the use of silver salts and proteinates started spreading in medicine. It can be described as either localized or generalized. Normally localized argyria is caused by external contact with silver, both occupational and medicinal, and can be developed in the areas of treatment, especially on the hands and the eyes, as round- or oval-shaped grayish spots. On the contrary, generalized argyria results in a widespread pigmentation following silver ingestion or massive absorption through mucosal surfaces. In this way silver is assimilated, carried by the bloodstream and deposited in various tissues throughout the body, so that even internal organs become pigmented, together with extended portions of the skin, nails and eyes. In the skin, silver can widely deposit as dark brown-blackish extracellular granules in the upper dermis and between collagen bundles, while in the intestinal mucosa such granules have been found both in the lamina propria and at the basement membrane of duodenal epithelium.[51] Silver granules have also been identified in the brain of patients suffering from argyria, concentrated in the choroid plexuses and leptomeninges, as well as in the walls of many intraparenchymal vessels, especially of the hypothalamus, cerebellum, substantia nigra, and basal ganglia.[20] Neurological symptoms, like seizures, are an unusual consequence of silver toxicity, but nonetheless there are a few cases reported.[52-54]

Although it has also been recently described in silver nitrate makers following occupational exposure, generalized argyria was more common in the past, when colloidal or protein silver were used to treat a number of pathologies and infections. In fact, this condition is normally connected to the long-term ingestion or application of silver-containing medicines. However, the currently increasing popular interest towards colloidal silver in alternative to standard antibiotics and as a cure-all remedy will surely generate a new surge of argyria cases, like the recent one of a man who turned blue-grey after a ten years of drinking of his home-made colloidal silver solutions to treat dermatitis. The man died

in 2013 after suffering a heart attack and stroke, which have been declared as unrelated to his skin discoloration, as reported by many newspapers. In spite of the fact that a number of studies considering silver toxicity in humans have stated it has no effect on the cardiovascular system,[55] these observations have been based on samples of workers occupationally exposed to silver (both soluble and insoluble) by inhalation or contact, and to medicinally/therapeutically exposed patients, but none of the subjects had such a long history of daily ingestion of colloidal silver as the person cited above, who indeed experienced a massive accumulation of this metal in his body tissues. It is known that iron overload and accumulation in the heart of thalassemia major patients have been recognized as a cause of heart failure[56-58] due, together with other biological mechanisms, also to conduction delay and subtle repolarization abnormalities with consequent arrhythmias, diastolic and systolic dysfunction.[59,60] It cannot be excluded that extensive accumulation of any other metal in the heart can lead to similar impairment of electrical signal conduction, and this aspect should be further investigated.[61]

Silver nanoparticles (AgNPs) are another form of silver widely employed in medicine in the past century, and now rediscovered as an alternative to standard antibiotics. Their toxicity to humans will be discussed thoroughly in a dedicated chapter (see below), but, in spite of many studies declaring this nanomaterial as harmless to mammalians, there is increasing evidence that this may not be the case,[62] and further research should be dedicated to settling the dispute once for all.

AgPNs can also be produced in the silver industry with consequent exposure of workers to the risks associated with inhalation or skin contact, especially linked to argyria: threshold limits are yet to be determined for silver nanopowder exposition as well.

Following the data collected during the past years by a large number of studies, metallic silver has been acknowledged as less toxic compared to its soluble salts, and the American Conference of Governmental Industrial Hygienists has indicated distinct threshold limit values for metallic silver (0.1 mg/m^3) and soluble compounds of silver (0.01 mg/m^3). However, the permissible exposure limit recommended by the Occupational Safety and Health Administration and the Mine Safety and Health

Administration, and the recommended exposure limit established by the National Institute for Occupational Safety and Health, converge towards the value of 0.01 mg/m^3 for all forms of silver.[55]

2. Silver in medicine

2.1 A bit of history

The employment of silver bowls to maintain water and other beverages pure for long periods was a common practice in ancient civilizations, such as the Greeks, as reported by Herodotus, or the Romans who kept wine in silver containers to avoid moldering. The empiric knowledge that such practice could prevent festering and decomposition probably led to the custom of using silverware and cutlery by the wealthy throughout time.

In the era of explorations and the conquest of new territories, such as Australia and the American "Wild West", settlers and pioneers adopted a similar habit to avoid spoilage, by inserting silver tableware or coins into their water or milk barrels.

Silver has been exploited in medicine with different applications. In the early 1800s, doctors sutured surgical wounds with silver wires, and a silver leaf was applied onto wounds occurred to soldiers during World War I to avoid infections and facilitate healing.

Colloidal silver was extensively employed in the medical practice at the beginning of the 20th century, for instance as a germicide in hospitals. Prestigious medical journals described the efficacy of silver colloidal dispersions as bactericides with no adverse drawbacks. For instance, in 1918 a paper by T. H. Anderson Wells appeared in the Lancet reporting that a preparation of colloidal silver was "used intravenously… without any irritation of the kidneys and with no pigmentation of the skin".[63] The use of such a remedy, which seemed to be both effective and safe, decreased in popularity during the antibiotic era, although many physicians continued prescribing it as nose drops against colds and allergies, or as eye drops to heal ophthalmic infections. Lately, it has regained popularity as an "alternative" drug against a number of pathologies and diseases, most of which are only alleged and not verified by sound scientific findings.

Silver salts have been employed in the past as antibacterial agents against infections such as conjunctivitis, gastroenteritis, gonorrhea and syphilis, but also to treat mental illness and nicotine addiction. The Merck Index First Edition (1889) listed at least 18 silver salts for pharmaceutical purposes. Silver nitrate was indeed the most diffused one. It was called *lapis infernalis* (or lunar caustic by the alchemists) and its earliest applications date back to 69 B.C., when it was first described in the Roman Pharmacopeia.[35] In more recent times and for several decades a 2% solution of $AgNO_3$ was the only effective treatment for neonatal conjunctivitis (i.e. ophthalmia neonatorum), after Credé introduced this method in 1881, which was commonly used in Africa until a few years ago. Silver nitrate has also been applied in the treatment of burns as a 0.5% solution,[64] or in cutaneous wart eradication with discrete success.[65]

Silver proteinates, also known as mild silver proteins, once occupied an important place in medicinal practice. Protargol, usually sold as 8% silver in combination with albumin, is currently employed in electron microscopy as a positive stain for carbohydrates or in light microscopy to stain nerve tissue, but its place in medical history can be found as the treatment for gonorrhea before the introduction of antibiotics. The first silver protein formulation was invented by the German chemist Arthur Eichengrün, and entered the therapeutic practice in 1897.[66] Argyrol is another example of silver proteinate; it was introduced against gonorrhea in 1902 by Dr. Albert Coombs Barnes and the German scientist Hermann Hille, and it is still in use today. Argyrol was also used to counteract local infections in mucous-membrane-lined organs, or to avert gonorrheal blindness and other ophthalmic conditions in newborns. Other silver proteinate complexes have been listed and briefly described by Squire's Companion to the 1916 British Pharmacopoeia, and are reported in table 1.

<TABLE 1>

Silver sulfadiazine is a complex in which an Ag^+ ion is bound to a sulfonamide antibiotic. It was discovered in the 1960s and soon employed as a topical drug in the treatment of burn wounds, and less frequently to manage skin wounds in general. Its current way of administration is in the form of

a 1% cream, as an auxiliary therapy to avoid or treat wound sepsis in second- and third-degree burns after resuscitative measures (e.g. management of electrolyte disturbance or control of shock and pain). A 1% cream formulation of silver sulfadiazine and enrofloxacin is commonly employed in the veterinary practice as an antibacterial-antimycotic emulsion. Silver sulfadiazine is included in the World Health Organization's List of Essential Medicines and still commercially available and recommended, regardless of a long lasting diatribe on its efficacy and drawbacks. In fact, the effectiveness in promoting wound healing or preventing wound infections, especially those linked to burn injuries, was questioned by two Cochrane systematic reviews and studies from 2010 and 2013, respectively, which concluded that the evidence collected was not sufficient to establish silver sulfadiazine efficacy in such treatments. [67,68] Other reviews followed, all claiming that the quality of the trials on silver sulfadiazine was limited, and new studies evidenced possible side effects in the administration of this drug, more severe than the mild local effects like burning, itching, pain, rash at the application site, skin discoloration and those connected with the sulfamidic antibiotics (all also reported by the manufacturers in the prescribing information). Leucopenia, for instance, seemed to be a significant drawback[69,70] together with bone marrow toxicity.[71] Nevertheless, also these findings have been put under scrutiny,[72,73] leaving the question controversial. In spite of all disputes, and although controlled comparative studies are lacking, silver sulfadiazine is still considered by many clinicians as one of the indispensable topical anti-infective drugs in burn patients[74-77] and its further applications in treatment of burns or infection prevention are currently under study.

Silver arsphenamine was first used at the beginning of the 20th century against syphilis,[78,79] after the successful employment of uncomplexed arsphenamine (also known as Salvarsan or compound 606, an organoarsenic species considered to be the first chemotherapeutic drug) as an antisyphilitic agent in the early 1910s. Its fortunes lasted only a couple of decades, then penicillin came and supplanted the use of arsenic compounds in the treatment of tripanosomiasis. Moreover, a number of cases of argyria amongst patients treated with silver arsphenamine may have accelerated its decay in the medical practice.[35]

Silver acetate is mainly used as a pesticide, but it has also been employed in gums, sprays, and lozenges to dissuade smokers from smoking. It appeared for the first time in Europe as an over-the-counter smoking-deterrent lozenge at the beginning of the 1970s, while a few years later it was also sold in the form of chewing gums. When silver acetate comes into contact with smoke, it produces a repulsive metallic taste in the smoker's mouth, thus discouraging cigarette consumption.[45,80] When administered as lozenges containing 2.5 mg of silver to a cohort of 500 adult smokers for a three-month period, this compound reported only a "modest efficacy". When the period was extended to 12 months, any prevention failed.[46] A Cochrane review[81] pointed out that the existing trials demonstrated limited evidence for a specific effect of silver acetate in favoring smoking cessation, and that the effectiveness of its preparations could be lower than nicotine replacement therapy, probably due to the fact that an unpleasant stimulus is not an effective smoke deterrent. Argyria and convulsive seizures developed in a patient that had been addicted to silver acetate anti-smoking pills for 40 years.[52] Similarly, a case of argyria in a woman who had assumed massive doses of anti-smoking lozenges containing silver ethanoate over a period of 2.5 years was reported in 1980. Symptoms of argyria appeared after the first 6 months of exposure. A whole body neutron activation analysis evaluated her total silver load to be around 6.4 (plus or minus 2) g.[82]

2.2 Silver in medicine today

Several silver-based drugs employed in the past, as it has been pointed out above, are still over-the-counter and commonly prescribed by doctors for their conventional uses. Nevertheless, some of them are currently being improved or tested for novel applications. For instance, silver fluoride has raised new interest in the dental practice against caries[83,84] and in the treatment of hypersensitivity in teeth;[85] silver nitrate has been tested in the treatment of cysts and abscesses[86,87] or in antifungal trials, being active against two mycotoxigenic strains of pathogenic species (*Aspergillus flavus* OC1 and *Penicillium vulpinum* CM1)[88] and ocular fungi (*Fusarium* spp. and *Aspergillum* spp.).[89] Silver ions

have been incorporated into catheters and cloths for surgical wound dressing to prevent infections, or into textiles for the treatment of acute neurodermitis; silver alginate has been tested in the prevention of central line infections through the use of catheters in very low birth weight infants.[90] Meanwhile, novel silver compounds or formulations have emerged as promising drugs for future treatments. Silver nanoparticles are taking the lion's share in the medical field and other technological domains, but silver complexes are also drawing interest for their antimicrobial and chemotherapeutic properties.

The mechanisms through which silver exerts its toxic effects against bacteria, fungi, protozoa, and cancer cells are rather complex and have started being elucidated only recently. They depend on both the type of silver compound involved and its cellular target. However, it seems that the biologically active species is always its cationic form, Ag^+, whether released in the organism from silver salts, complexes, or nanoparticles.

The cytotoxic mechanisms of silver ions are based on a series of damages caused by Ag^+ to the bacterial or cancer cells:[91,92]

- Ion exchange impairment: Ag^+ inhibits phosphate uptake and exchange causing accumulation of this anion, favors the release of K^+ and knocks off proton motive force through the cytoplasmic membrane, leading to cell death.

- Complex formation with DNA and RNA: Ag^+ is able to bind nucleic acids (rather than the phosphate moiety) in a very efficient way, so that cell replication processes can be disrupted.

- Enzyme inactivation and protein denaturation: Ag^+ ions are able to strongly interact with peptides and proteins, forming complexes with their donor groups, especially with thiol and phosphate moieties, but also with carboxylate, hydroxyl, amino, imidazole, and indole groups. Such binding may change the protein or enzyme structure causing impairment of its function, especially in the enzymatic oxidations of fumarate, glycerol, glucose and succinate, but also affecting lipoxigenase[93,94] and the selenoenzyme thioredoxin reductase.[95] Moreover, silver effectively binds to reduced glutathione, as previously pointed out. Finally, it is able to disrupt iron-sulfur clusters.[96]

- Shrinking and breaking of cell and mitochondrial membranes: the interaction between silver and the cell walls can lead to structural membrane modifications. Ag^+ is able to coordinate thiol groups of proteins and enzymes found on the cellular surface, causing destabilization of the cellular membrane and a breakdown of the ATP synthetic processes. The electrostatic attraction between Ag^+ ions and the negatively charged cell membranes of bacteria leads to a binding of the cation to the phospholipid bilayer and induces a massive leakage of protons.[97] Ag^+ ions can also damage the cytoplasmatic membrane integrity and permeability by removal of an electron from cellular constituents and enzymes.[98] Furthermore, silver is able to disrupt mitochondrial homeostasis, causing its imbalance and membrane depolarization.[99-101]

- Promotion of VBNC (Viable But Not Culturable) bacteria: Ag^+ causes microorganisms to fall in a state of very low metabolic activity and division impairment. Cells in this state get smaller in size because they cannot rely on a normal routine for nutrient respiration, transport, and synthesis of macromolecules. This condition can keep the cell life suspended even for months, but it usually leads to its death.[102]

- The "zombie" effect: silver-killed and isolated bacteria are able to induce death in viable bacteria they come into contact with. The dead bacteria, having internalized Ag^+ ions, behave as a reservoir able to transmit silver ions to the live bacteria, also transferring their toxic action to them[103] (Fig. 2).

< FIGURE 2>

The antibacterial efficacy attributed to AgNPs is higher with respect to silver salts and complexes for different reasons.[92] First, silver nanoparticles have an extremely large surface area, so that they can exert better contact with the surface of bacteria. Upon adhesion to the membrane wall, AgNPs cause its depolarization, followed by a loss of integrity which in turn leads to impaired transport, interruption of energy transduction, imbalance of respiration, and finally cell lyses and death, as

previously seen. Moreover, silver can penetrate through the holes in the membrane and interact with sulfur-containing intracellular components, such as proteins and enzymes.

Second, AgNPs work as an effective Ag^+ reservoir: once the nanoparticles are internalized by the cell, a gradual oxidation of the silver atoms on such an extended surface is able to release the biologically active species in a continuous way, providing a constant flux of silver cations that can attack the bacterial targets more efficiently for a prolonged period.

However, the release of silver ions alone cannot explain the higher efficiency of AgNPs against bacteria compared to other silver compounds. Production of Reactive Oxygen Species (ROS) has been suggested as an explanation, specifically as a mechanism catalyzed by silver oxidation in a Fenton-like reaction.[104,105] ROS, in the form of radicals such as singlet oxygen ($^1O_2$), can be generated in the cell membrane, thus producing oxidative stress and impairment due to a series of events, from lipid peroxidation and alteration of proteins to inhibition of enzymes (especially ATP-ases), and RNA and DNA disruption or mutation, leading to irreversible damage to DNA replication mechanisms, which consequently disturb cell division and metabolic processes.[106]

Moreover, another interesting mechanism behind AgNPs antibacterial action has been evidenced in the case of nanocrystalline silver (NCS) particles. The increased surface area of NCS permits a more extended surface oxidation by atmospheric oxygen, with consequent formation of silver oxide $Ag_2O_{(s)}$. Such a process creates a reservoir of available silver ions $Ag^+_{(aq)}$ and the release of hydroxide $OH^-_{(aq)}$ upon contact with an aqueous fluid, such as the wound bed where NCS are commonly employed. The combined action of the Ag^+ species and the alkaline pH results in a synergistic effect, leading to a fast and highly efficacious activity against micro-organisms.[27]

To sum up these findings, the antimicrobial behavior of AgNPs (Fig. 3) can be imputed to the following mechanisms:[107]

- Adhesion of AgNPs onto the surface of the cell and rupture of the membrane;

- Internalization of the nanoparticles and impairment of intracellular organelles (mitochondria, vacuoles, ribosomes) and biomolecules (proteins, enzymes, lipids, and DNA);

- Induction of cellular toxicity and oxidative stress by ROS and free radicals;

- Modulation of signal transduction pathways;

- Modulation of the human cells immune system by triggering the inflammatory response, which further promotes inhibition of microorganisms.[108]

<FIGURE 3>

The complexity of such mechanisms indicates that silver toxicity is hardly liable to the development of resistance in bacteria, a phenomenon which is instead very common in classic antibiotics. For this reason, bacterial resistance to silver is rather uncommon and often transitory.[109,110] The literature presents one report of a silver-resistant strain of *P. stutzeri*, which has been found in a silver mine,[111] while three silver resistance genes have been identified in *E. coli* isolates with extended-spectrum beta-lactamases of the CTX-M type.[112] Moreover, resistance to AgNPs has just been shown by a recent study where Gram-negative strains of *E. coli* 013, *P. aeruginosa* CCM 3955 and *E. coli* CCM 3954 developed it after repeated exposure. The reason behind this lies in the production of an adhesive protein, flagellin, a globular protein forming the filament in a bacterial flagellum, which is also able to start AgNPs aggregation, thus reducing their colloidal stability and consequently their antibacterial activity. This is not an adaptive behaviour involving genetic changes, but only phenotypic modifications, and it can be easily counteracted by inhibiting flagellin production.[113]

In spite of these findings, it is possible that the clinical incidence of silver resistant bacteria remains low because silver, unlike common antibiotics, as previously seen activates multiple mechanisms and hits different targets within the bacterial cells[110] so that it becomes very difficult for these microorganisms to develop adaptive countermeasures. Moreover, there is no evidence that silver can confer cross resistance to antibiotics.[114]

The outstanding lethal effects of silver on bacteria and other lower organisms do not correspond to a high toxicity in humans. On the contrary, a number of studies have evidenced that silver seems to be

rather safe for mammals, although several others are showing a different point of view. However, in general the benefits outweigh the risks. Thus, by considering the association of high antibacterial properties with low toxicity in humans, the renewed interest in silver-based antimicrobial drugs can be easily explained.

3 Silver Compounds

3. 1 Silver complexes

Ag(I) coordination compounds have been recognized as promising therapeutics due to their outstanding biological properties. In fact, they show antibacterial, antimycotic, antiparasitic, anticancer[1,2,91], and antimalarial[115] activity, as evidenced in a high number of studies.

The efficacy of silver complexes against bacteria and cancer cells depends on a number of factors: lipophilicity, redox proclivity, water solubility and stability, and rate of release of the silver ions. These factors are strictly controlled by the characteristics of the ligands and their requirements in both steric and electronic properties. Finally, it must be considered that even a well-designed Ag(I) complex for medical use can lose part or all of its activity when transferred to *in vivo* conditions, due to the formation of insoluble AgCl or binding of the complex to cell enzymes. This problem could be solved by the incorporation of the silver drug into biodegradable or biocompatible nanoparticles for transportation and delivery.

Silver complexes for therapeutic purposes have been prepared with a vast variety of ligands (Fig. 4), but those scoring the best results usually contain N-heterocycles (phenanthrolines, pyridines and polypyridines, etc.), N-Heterocyclic Carbenes (NHC), and phosphines.[91,116] Another popular approach in the synthesis of successful metal complexes is to associate the action of the metal center to that of a drug that is already in use or has already exhibited therapeutic properties as such. In this way the synergistic effect of the two components is expected to enhance the overall performances of the resulting complex, although this might not always happen. Commonly both antibiotics (vancomycin, metronidazole, sulfachloropyridazine, sulfamoxole, etc.) and natural drugs or their

derivatives (isonicotinic acid, salicylic acid, coumarin, etc.) have found application in this field with interesting results.[91]

<FIGURE 4>

Silver coordination compounds are effective against various bacteria, fungi, protozoa, and several cancer cell lines, evidencing in the latter case anticancer mechanisms that share the same biological pathways as the antibacterial ones, and for these reasons are completely different from those at the basis of cisplatin cytotoxicity.[91,117]

The efficacy of silver compounds as anticancer agents *in vitro* is well documented together with their low cytotoxicity,[1,91] but the information about their *in vivo* activity is lacking or rather scarce. To date and to the best of our knowledge, only a few studies have been reported on the effects of silver complexes on tumor (e.g. ovarian cancer and non-small-cell lung carcinoma) xenograft murine models,[118-120] resulting in decreased growth of the tumor mass or in cell death. Silver compounds containing NHC ligands are the most active and promising in this respect, although a silver acetate carbene complex with remarkable activity *in vitro*, when tested *in vivo* on CAKI-1 tumor-bearing NMRI:nu/nu mice[121] showed almost no effect on the neoplastic formation, but was rather toxic to the host, with body weight loss and eventually death. Nevertheless, nearly nothing is known about possible side effects that could develop after human administration of Ag(I) complexes, although silver *per se* is still considered to be non-toxic for humans and other mammalians. Another point is the estimated bioavailability for Ag(I) ions incorporated into coordination compounds: such values appear to be relatively low; even so, they seem to be slightly higher than analogous Pt(II) or Au(I) species *in vitro*. Given the nature of silver ions, though, their bioavailability could further drop *in vivo* due to precipitation as insoluble AgCl or sequestration as Ag-protein complexes.

In the field of old drugs with new applications, silver sulfadiazine found a place as a preventive agent for catheter-related infections, showing promising results as such or in association with other

"conventional" antibiotics, like chlorhexidine, especially in critically ill patients.[122-124] Moreover, its efficacy in the treatment of burn wounds has been enhanced by new technological developments in the area of nanotechnology and new materials. Thus, silver sulfadiazine has been loaded on microsponge-based gels to decrease the frequency of application of this drug and skin irritation, at the same time achieving low cytotoxicity on skin cells and enhanced wound contraction;[125] when loaded on bacterial cellulose/sodium alginate composite films, it improved its antibacterial properties and biocompatibility;[126] silver sulfadiazine-loaded chitosan/chondroitin sulfate films showed a good antibacterial activity against *P. aeruginosa* and *S. aureus* while they were not toxic to mammalian cells, indicating their potential as an effective wound dressing material;[127] finally, silk fibroin nanofibers are commonly used as scaffolding material for skin regeneration, and when impregnated with silver sulfadiazine they have been associated with faster wound healing compared to other commercially available wound dressing; some cytotoxic effects have been evidenced as well.[128] These interesting results indicate that even an old and much-discussed drug like silver sulfadiazine, when reinvented by using new materials and technologies can regain life and display improved performances.

Compared to "classical" antibiotics, silver compounds emerge for their remarkable activity against biofilms. Biofilms are one of the two growth modes for bacterial (but also fungal and microalgal) cells: one is the planktonic state, in which the micro-organisms are freely suspended as single cells in the aqueous media, while the second is under the form of sessile aggregates, able to adhere both to living or non-living surfaces.[129-131] Such colonies can be composed either by a single species or different types of micro-organisms surrounded by a self-generated matrix of extracellular polymeric substances (EPS), formed by an aqueous solution (up to 97 % is water) mainly containing polysaccharides, but also a small quantity of proteins and enzymes, DNA, and RNA. One of the main biological features of biofilms is that they are formed by microbes in response to many stimuli, ranging from cellular recognition of specific or non-specific anchoring sites on a surface (either living or inert), to nutritional signals, or even exposure of planktonic species to sub-inhibitory levels of

antibiotics.[132,133] When a cell shifts from the planktonic to the sessile mode of growth, large sets of its genes start to be differentially regulated so that the cell undergoes a phenotypic change in behavior.[134]

The typical example of a biofilm is dental plaque, or the biofouling on seagoing vessels, but it has been recently found that bacterial aggregates are also involved in many infective processes, and according to the National Institutes of Health about 65% of all microbial infections, and 80% of all chronic infections are associated with biofilms.[135] Moreover, it was also evidenced that biofilms are present on the surface of medical devices, including contact lenses and catheters, and this becomes critical when considering that the implantation of biomedical devices is connected to about 60-70% of all nosocomial infections.[136] Finally, it was evidenced that the EPS matrix is hardly permeable by antibiotics and human immune system cells, so that bacteria in their sessile form are 100 to 1,000 times less-responsive to antibiotics than planktonic species, thus representing a serious danger to public health.

Among the complexes showing promising activity against biofilms there is, again, silver sulfadiazine which was screened against mature *P. aeruginosa* biofilms as one of the most common conditions associated with burn wounds.[137] This study evidenced that the concentration of silver required to destroy the biofilm was 10-100 times higher than the dose effective on planktonic forms, highlighting the resistance of bacterial aggregates to standard treatments and the need for more accurate protocols for the complete elimination of bacterial infections so frequent in burn wounds.

Another interesting group of Ag(I) complexes with biological properties is based on NHC ligands, which have been screened against *Listeria*, *Pseudomonas*, *Staphylococcus*, and *Escherichia* strains.[138,139] This research identified lipophilic Ag(I) species possessing aromatic groups on the NHC ligand as the most efficient at inhibiting biofilm formation.

At last, a very effective compound against biofilms is the unusual silver oxynitrate, $Ag(Ag_3O_4)_2NO_3$, a mixed complex in which oxygen atoms concur in the stabilization of both the Ag(II) and Ag(III) oxidation states, helping them coexist steadily at room temperature. Silver compounds with high

oxidation numbers are rare in biological studies due to their instability; nevertheless, for the same reason they can exert their activity against bacteria in a more efficient way. Silver oxynitrate antimicrobial action has been screened against *P. aeruginosa* and fluoroquinolone-resistant *P. aeruginosa* (FQRP), *E. coli* and uropathogenic *E. coli* (UPEC), *S. aureus* and methicillin-resistant *S. aureus* (MRSA), *C. albicans* (ATCC 14053) and *C. tropicalis*. Oxysalt, compared to other silver compounds such as Ag_2SO_4, $AgNO_3$, AgO, Ag_2O or silver sulfadiazine, is able to eradicate biofilm and planktonic populations of the examined strains at lower concentrations than those of the other tested metal salts.[140] Another study revealed that silver oxynitrate can release high amounts of Ag ions, including Ag^{2+} and Ag^{3+} species, with no influence on the pH of the medium, contrarily to nanocrystalline silver dressings (*vide supra*), and that this compound has a long lasting killing effect on antibiotic-resistant bacteria originally isolated from cutaneous wounds, including vancomycin-resistant *enterococci* (VRE), methicillin-resistant *S. aureus* (MRSA), carbapenem-resistant blaNDM-1-positive *K. pneumoniae* and blaVIM-2-positive *P. aeruginosa*, grown both planktonically and in a biofilm, with 75% reduced silver doses. It has also been demonstrated, via biocompatibility tests, that $Ag(Ag_3O_4)_2NO_3$ is safe for cytotoxicity, acute systemic toxicity, irritation and sensitization, opening the path to its use in the medical practice.[141] The same silver oxysalt has demonstrated its efficacy not only on single species biofilms, but also on dual bacterial assembles, a closer model to natural biofilms, where the coexistence of multiple species in the bacterial community is the normal occurrence.[142]

While the toxicological profile of orally administered silver compounds has yet to be fully defined, topical application for the treatment of chronic wounds remains the preferred treatment, as does for these new drugs under trial.

3.2 Silver nanoparticles

The future of metals in medicine seems strictly connected to their nanosized forms. Nanotechnology is in fact showing incredible potential in both therapeutic and diagnostic applications, so that the word

"theranostics" has been coined to combine these two functions. Nanomaterials of organic, inorganic and mixed origin can be employed as drugs, carriers, probes, imaging contrast media, biosensors, etc. but they are also rapidly spreading in the healthcare and cosmetic fields, even as consumer products. The Nanotechnology Consumer Products Inventory (NCPI) has launched a Project on Emerging Nanotechnologies in 2005, in an effort to create a worldwide catalog of nanotechnology-based products and help citizens, customers, governments and business anticipate and manage possible health and environmental consequences of nanotechnology. The first inventory listed about 54 products in 2005, but they were already 1012 in 2010 and 1814 in 2013, from 622 companies in 32 countries.[143] Apart from the silver industry, the main applications were in health and fitness (762 products, 42%), as antimicrobial, antifungal agents, or dietary supplements; cosmetics (sunscreens, toothpaste, deodorants, makeup powders and antiaging creams); textiles (antibacterial and antifungine agents, antistain and antiwrinkle clothing); and food (preservatives, antibacterial and antifungine agents in chewing gums, mints, candies, frostings, etc.). Silver was the most used metallic nanomaterial, present in 435 products (24%), although often the declared content in total silver does not match the measured values.[144]

Nanomaterials are regulated without specific provisions in the U.S. as hazardous chemical substances and pesticides, under the EPA's Toxic Substances Control Act, while the Federal Food, Drug, and Cosmetic Act regulates their use as food additives, drugs, or cosmetics. Once a substance or compound is declared as "safe" in its bulk form, there are no further controls on the same material in its nanosized derivatives, but it is known that nanoparticles have completely different behaviors compared to their bulky counterparts. Silver is not an exception. AgNPs, commonly deemed as harmless to humans, may have adverse effects on lower organisms (especially for aquatic life) and evidence is emerging that they can also be dangerous for mammalians, humans included. Nevertheless, they have demonstrated outstanding properties in the medicinal field and encouraging results as therapeutics. Currently, more than 100 silver-containing medical devices have been approved for use by the FDA.

AgNPs have been prepared most frequently as metallic silver, but also as AgCl and Ag_2O compounds. Their properties vary according to their composition, dimension, size, shape, presence of capping agents and coatings, surface charge, concentration, and colloidal state, so comparing their results as therapeutics is rather difficult.

3.2.1 Metallic AgNPs

Colloidal silver is a suspension of nanometric metallic silver in water and, as previously mentioned, it has been widely used in medicine in the past century before the advent of antibiotics. Since then, the interest in silver nanoparticles has experienced a quick decline, only to regain appeal as the phenomenon of multiresistance to antibiotics became a major concern for public health. Experimentation with AgNPs began on a wide range on microbes, fungi and parasites, together with new ways of production which were more effective, less expensive, higher in yield and eco-friendly. AgNPs can be synthesized via chemical or physical techniques. The latter include UV irradiation, evaporation/condensation or aerosol technologies, lithography, laser ablation, ultrasonic fields, and photochemical reduction. In spite of the fact that physical methods do not involve toxic chemicals, usually have fast processing times and produce nanoparticles in a narrow size distribution range, they are highly energy-demanding and cannot be used on a large production scale.

Conventionally, AgNPs are chemically prepared by reduction of metal salts to obtain pure metal nanoparticles, and then using stabilizing or capping agents to prevent their aggregation into clusters. The most common synthetic strategy involves the chemical reduction of silver nitrate or silver tetrachloride by borohydride, citrate, ascorbate, or hydrogen gas to produce stable colloidal dispersions in solvents.[145] Stabilizers are surfactants and ligands or polymers containing functional groups such as poly(methacrylic acid), polyvinylpyrrolidone, poly(ethylene glycol), poly(methyl methacrylate).[146] This typical procedure is fast and efficient, but during the formation of nanoparticles a number of toxic chemicals (reactants, solvents and stabilizers) can be absorbed onto their surface, making them unsuitable for medical applications. Recently, “green” alternatives have been introduced

to the classical chemical synthesis, which include biogenic methods using plants, bacteria, fungi, viruses and yeasts extracts as solvents, reducing/capping agents, and/or stabilizers.[147-149] Such procedures (Fig. 5) are easy, clean, reliable, nontoxic and eco-friendly. The method is very simple: the crude extract (either intracellular or extracellular) of the chosen organism is just mixed with a solution of the metal salt at room temperature. The reaction is complete within minutes, when the reduction is non-enzymatic, or can take longer (between 24 and 120 hours) in case of enzymatic processes.[146]

The general reaction, however, can be written as:

$Ag^{I}NO_3$ + phytochemicals → Ag^{0}NPs + byproducts

In the case of enzymatic reaction, the reducing agent is probably a nicotinamide adenine dinucleotide phosphate-dependent reductase. The nonenzymatic reaction, on the other hand, proceeds thanks to other microorganism components (polysaccharides, proteins, cofactors) or phytochemical molecules that also behave as stabilizing agents.

Another hypothesis for nanoparticles formation is that Ag^+ ions bind to the surface of proteins present in the plant extracts and are reduced by the same proteins, leading to their secondary structure change and formation of silver nuclei. The silver nanoparticles successively grow by further reduction of Ag^+ ions and deposition on silver nuclei.[150]

Plant fluids are the most used reducing media in the bio-synthesis of nanoparticles. Leaves extracts are the most frequently employed, but so are also bark, aerial parts, fruits (whole or peeled), flowers, roots, tubers, rhizomes, latex, moss, bulbs, heartwood, gums, callus and seeds. The phytochemicals responsible for silver reduction by plant fluids can be virtually infinite, including flavonoids, terpenoids, proteins and enzymes, sec-alcohols, polyphenols, phenol hydroxyl and carboxylic groups of arabinose and galactose, phenolic glycosides, reducing sugars, aliphatic and aromatic amines, alkaloids, water-soluble heterocyclic compounds and saponins.[151]

There are four factors influencing the synthesis of AgNPs based on plant extracts which also affect the particles size:[151-155]

- pH. A variation in pH alters the charges of the biomolecules in the extracts which can interact in different ways with the Ag^+ cation, thus determining the properties of the AgNPs produced.

- Temperature. Generally, the reaction rate increases as the reaction temperature rises. In high-temperature reactions, thermally stable compounds can lead to higher yields.

- Reaction times. By increasing the reaction time, the reaction rate also normally increases.

- Ratio of plant extract to silver nitrate. This is a very important factor, since it can influence both AgNPs' size and shape.

<FIGURE 5>

The diameter of the produced polydispersed AgNPs ranges between 5 and 200 nm.[148] Moreover, silver nanoparticles display different activity and cytotoxicity depending upon the medium in which they were prepared, since the latter determines size, shapes and capping agents, and reactivity as well (Table 2).[156]

AgNPs produced via plant-mediated biosynthesis have shown impressive potential against a number of pathogens, including bacteria (both Gram-positive and Gram-negative), fungi, protozoa, mites and larvae. They have also been effective against several cancer cell lines while showing low or no toxicity to normal cells.[157] Moreover, their high stability leads to potential applications in chemical sensing, biological imaging, gene silencing, and drug delivery.[158]

Bacterial culture supernatants have also been employed in the synthesis of AgNPs with fair success. The microorganisms used in these procedures were numerous and included, amongst the others, *B. cereus*, *B. subtilis*, *B. licheniformis*, *E. coli*, *E. cloacae*, *K. pneumonia*, *L. acidophilus*, *S. aureus*, and *P. aeruginosa*.[158] While the method is easy, the mechanisms behind such extracellular synthesis are not known and are susceptible of genetic modifications. In one case, a study conducted on the

supernatant of *Ochrobactrum rhizosphaerae*, which was able to produce spherical AgNPs effective against cholera, identified a glycoprotein exopolymer as the responsible agent for the synthesis and capping of the nanoparticles.[159]

<TABLE 2>

Finally, a number of studies have reported of natural polymers such as chitosan, starch and tannic acid behaving as reducing agents in the biogenic synthesis of AgNPs, which worked rather well.[149,160,161]

Another "green" approach for the preparation of AgNPs is the photoinduced or photocatalytic reduction of silver ions using carbon dots as reducing and stabilizing agents. This is a one-step synthesis in which the application of commercially available LED lights significantly decreased both reaction time and energy consumption.[162]

3.2.2 AgNPs toxicity to microorganisms: size- and shape-dependent activity

Silver nanoparticles toxicity initially seemed to be only ascribed to the release of Ag^+ ions, the bioactive agents, but this was just a simplified view of the process. In fact, a relationship between physicochemical characteristics of silver nanomaterials and their degree of toxicity soon became evident. Shape, dimension and other specific features depending on the way they are synthesized are the main factors controlling their antibacterial efficacy. AgNPs interact with bacteria, viruses and fungi in a shape-dependent manner.[163-165] For these reasons, silver nanoprisms, rods, sheets, beads, and mats have been synthesized and studied for their specific antibacterial action,[166] with nanobeads, nanocrystals, nanoplates and quantum dots as the most effective forms against bacteria. Another example is provided by the comparison of spherical or rod-shaped with truncated triangular shaped AgNPs, where the latter showed enhanced antibacterial action compared to the former.[167,168]

Moreover, size, surface coatings or capping agents, and surface charge have also been identified as important factors responsible for AgNPs efficacy.[166] For instance, it has been demonstrated that the smaller the nanoparticles, the higher the toxicity exerted, as reported in a recent study: when the size approaches the sub-10 nm range the activity is maximum, with 5 nm AgNPs scoring the fastest bactericidal action compared to 7 nm and 10 nm sizes.[169] On the other hand, a different study reported that decreasing the size of AgNPs increases their stability and biocompatibility.[170] A good compromise seems to be the range between 10 and 15 nm, where silver nanoparticles have higher stability, biocompatibility and antimicrobial activity.[171] AgNPs having such a diameter are approximately 100 times smaller than many bacteria (1-2 μm diameter) allowing many particles to anchor themselves to the surface of a single bacterium. However, AgNPs stop being effective above 50 nm, probably because their large dimension reduces the interaction with the bacterial surface and also impedes their internalization.

3.2.3 Antibacterial action

The most prominent application of AgNPs is against pathogenic bacteria, and this ability has been thoroughly discussed throughout the text in order to clarify their mechanism of action, so it will not be further debated. For a complete picture, however, it is noteworthy that silver nanoparticles have demonstrated their activity against an impressive number of bacteria, both Gram-positive and especially Gram-negative species, with a success depending on many factors (size, shape, concentration, surface charge, coating, etc.). Resistant and multidrug-resistant strains have also been tested, with positive and promising results.[172] Silver nanoparticles have been effective on biofilms as well.[173]

3.2.4 Antifungal action

Nanosilver (NPSs) is a powerful antifungal agent and its properties have been tested against a wide gamut of commonly diffused fungi. In a study, a number of 44 strains from six different yeast species

(*Candida albicans*, *Candida parapsilosis, Candida krusei, Candida glabrata,* and *Trichophyton mentagrophytes*) were screened, evidencing that NSPs are able to efficaciously inhibit their growth.[174] The exact mechanisms behind their antifungal action are still not clear, but it was found that AgNPs, similarly to their action with bacteria, can damage the fungal cellular membrane and impair the natural budding process.

3.2.5 Antiviral action

AgNPs have displayed antiviral properties against HIV-1, herpes simplex virus type 1, hepatitis B virus (HBV), Tacaribe virus (TCRV), monkey pox virus, murine norovirus (MNV)-1, recombinant respiratory syncytial virus (RSV), and influenza A/H1N1 virus.[27,146] Such antiviral activity seems to be higher than that of silver ions. The anti-HIV action of NPSs is probably due to the repression of the initial stages of the HIV-1 life cycle by nanoparticles binding to the disulfide sites of the CD4 (cluster of differentiation 4) domain on the gp120 glycoprotein and subsequent impairment of cluster differentiation of (CD)4-dependent binding, fusion, and infectivity. In this way silver nanoparticles are able to block both HIV-1 cell-free and cell-associated infection, behaving as a powerful virus killer.[175]

3.2.6 Anti-inflammatory action

Inflammation processes are mediated, above all, by cytokines. Excessive chronic production of inflammatory cytokines contributes to inflammatory conditions. AgNPs, especially in the form of nanocrystalline silver, have shown anti-inflammatory properties in both animal models and in clinical trials, by altering the expression of proinflammatory cytokines in a mechanism which involved the transformation of growth factor-α and tumor necrosis factor-α.[176] Moreover, nanocrystalline silver suppressed inflammatory cytokines and induced apoptosis of inflammatory cells in a murine model of allergic contact dermatitis.[177] Such an ability to reduce cytokine release, together with inhibition

of matrix metalloproteinases, decrease of lymphocyte and mast cell infiltration, and induction of apoptosis in inflammatory cells may be the key to explaining AgNPs' anti-inflammatory properties.

3.2.7 Anticancer action

Silver nanoparticles have been tested against numerous cancer cell lines, such as breast and lung cancer, hepatocellular, skin and oral carcinomas, and leukemia, with very promising results.[27] While the mechanisms behind their anticancer action are still under study, they appear to be connected with silver's ability to disrupt the mitochondrial respiratory chain, inducing the generation of ROS, and ATP synthesis, causing DNA damage, but also to its capacity to block cell cycle and activate apoptosis (*vide supra*), as evidenced for their antibacterial action.[178] AgNPs have been toxic not only to cancer cells but also to healthy ones, and this has been seen as a hurdle to their application in medicine. However, toxicity itself can be useful for cancer therapies, and so it is highly pursued: the example of cisplatin and its derivative can be very instructive in this sense.

AgNPs prepared via plant-mediated or other biogenic synthesis showed the most interesting proprieties since they were more active and less toxic compared to chemically synthesized nanoparticles, probably due to their capping.[157] New papers describing novel biosyntheses of AgNPs using the most disparate plant or bacterial extracts and evidencing their antimicrobial and anticancer activities appear in the literature with an impressive weekly frequency. Depending on the reducing organism involved, the part of the plant used in the extracts, the reaction conditions and the cancer cell line tested, different effects leading to cell death have been reported, such as disruption of membrane integrity, decreased cell growth, cytoplasmic condensation, cell clumping, cell shrinkage, and nuclear condensation and fragmentation (see for instance [179]), DNA laddering (see for instance [180]), caspase-dependent and mitochondrial dependent pathways.[181] One of the interesting results is that in most of the aforementioned studies, when healthy cells were treated with biogenic AgNPs they remained lively or only marginally affected compared to cancer cells. The reason for this behavior may be found in a pH dependent mechanism of AgNPs dissolution. In fact, silver as a bulk metal

does not dissolve in acids, while its nanosized particles do. In fact, colloidal biogenic AgNPs are able to release high amounts of silver ions in acidic pH, as verified by a series of experiments at different pH conditions.[182] The fact that tumor tissues exhibit acidic pH[183] may thus explain why cancer cells are more affected by AgNPs, namely due to a higher release of toxic Ag^+ in their cellular environment compared to healthy ones.

In any case, the promising results obtained with AgNPs in tumor cells *in vitro* should be soon validated *in vivo* to verify whether AgNPs can be a real alternative to cisplatin and their derivatives in the fight against cancer.

3.2.8 Toxicity to humans and mammalians

AgNPs can be cytotoxic not only to bacterial but also to eukaryotic cells.[184] Toxicity to mammalians and humans has been evaluated both *in vitro* and *in vivo*. *In vitro* experiments on a variety of cell lines have evidenced cytotoxic effects on human alveolar epithelial cell line, human peripheral blood mononuclear cells, neuroendocrine cells, murine and human alveolar macrophages, mouse germline cells, and rat liver cell line.[26,146] The mechanisms related to human toxicity are almost the same as those behind silver antibacterial activity and have already been discussed (see above).

In vivo toxicity has been evaluated mainly in cases related to medicinal assumption of nanosilver formulations.[185] In has been evidenced that, upon inhalation or systemic administration, low concentrations of nanometric silver (14.6±1.0 nm) soon arrive to the lungs and are subsequently translocated to the blood and other organs, such as liver, kidney, heart, and even brain.[186]

A similar destiny was evidenced in rats, where orally administered AgNPs were transferred to blood, kidneys, lungs, liver, testes, stomach, and again in the brain, with the additional indication that they did not induce any significant genotoxicity after administration of different doses of 60 nm AgNPs for one month.[187]

In general, there is no consensus on nanosilver toxicity to humans, and more research is needed to solve this long lasting dilemma.

3.2.9 Toxicity to guts microbiota

The human microbiota, residing in the intestine, is fundamental for immunologic, hormonal and metabolic homeostasis, so that it is now considered as an entire metabolic organ with multiple physio(patho)logical functions.[188] Gut microbiota is a complex and symbiotic ecosystem composed of several tens of trillion microorganisms, including bacteria, yeasts, viruses and archaea, living in a close association with the host. It is essential for vitamin synthesis, intestine maturation, local angiogenesis, regulation of enterocyte gene expression, and homeostasis of innate and adaptive immunity.[189,190] Silver has no or low toxicity for eukaryotic cells, but its antimicrobial action is not able to discriminate between "good" and "bad" bacteria, so that guts microbiota could be at risk following the ingestion of silver nanoparticles, which are present in a growing number of cosmetics and personal care products, but also in food and water. A decrease in gut bacteria population can lead to dysbiosis, a condition of microbial imbalance, which may induce tendency to develop pathologies and obesity. There are just a few studies on this topic, which have examined the influence of ingested AgNPs on gut microbiota, all but one agreeing on the conclusion that ingestion of silver nanoparticles at doses relevant for human dietary intake can cause microbial modification in the gut, affecting the different types of bacteria to a different extent.[191-194] The only discordant paper found out that an oral administration to rats of AgNPs of two distinct dimensions (20 and 110 nm) and coatings (polyvinylpyrrolidone and citrate) at the dose of 10 mg/kg body weight/day for one month did not modify the composition, diversity and structure of the murine gut microbiome. Both size and coating did not influence the results of this study. Thus, a basic difference between AgNPs and broad-spectrum antibiotics was evidenced, since repeat dosing of AgNPs, at rations corresponding to 2000 times the oral reference dose and 100-400 times the effective *in vitro* anti-microbial concentration, did not affect the murine microbiota.[195]

Given the limited number of studies carried out on such an interesting topic, it seems necessary to further investigate the aspects related to silver nanoparticles toxicity towards gut bacteria, especially in order to verify the possible effects of silver-induced dysbiosis, such as impairment of the immune system or obesity development.

3.2.10 Nanoparticles of ionic silver

Metallic silver nanoparticles and their ionic counterparts such as silver chloride (AgClNPs) and silver oxide (Ag_2ONPs) nanoparticles, have been prepared and tested for biological activity.

Their synthesis can either be based on chemical-physical (micro-emulsion or matrix-based technique, ultrasound irradiation,[196] etc.) or purely chemical methods, such as the precipitation of insoluble AgCl from an $AgNO_3$ solution with NaCl or HCl in the presence of stabilizers (i.e. polyvinyl alcohol,[197] chitosan,[198] etc.). Recently, as in the case of metallic AgNPs, its ionic forms have also been prepared via a biogenic synthesis. For instance, aqueous extract of plants (*Glycyrrhiza uralensis*,[199] *Morinda citrifolia*[200]) or algae (*Chlorella vulgaris*,[201] *Sargassum plagiophyllum*[202]) made AgClNPs available in an eco-friendly and time-efficient way. The silver chloride nanoparticles thus obtained have been tested for their biological properties, evidencing that they can exert antibacterial action not only on *S. aureus* and *E. coli*,[197] but also on *P. aeruginosa* and *S. enterica*,[199] and *K. pneumoniae*.[201] Moreover, Chitosan-stabilized AgClNPs in an ointment form were more effective than Vaseline as a wound-healing accelerator because of their improved antibacterial activity,[203] and AgClNPs dispersed on silk fibroin microfibers inhibited the growth of Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria.[204]

Metal oxide nanoparticles in general, but especially those containing silver oxide, have demonstrated significant antibacterial activity, as evidenced by several studies especially against Gram-negative microbes[205] and multiresistant strains,[206,207] but also as antileishmanial agents. The major concern emerging from these studies is that Ag_2ONPs, although highly efficient in killing bacteria and protozoa, can also be cytotoxic to eukaryotic cells and therefore to humans - hence the need of finding

new ways to decrease their toxicity for a safer use in medical applications. Again, the solution could come, also in this case, from the biogenic synthesis. Ag_2ONPs have been prepared using bacterium *Lactobacillus mindensis*,[208] leaf extracts of *Excoecaria agallocha*[209] or *Eupatorium odoratum*,[210] and root extracts of *Ficus benghalensis*.[211] Ag_2ONPs prepared in this way are not only effective bactericidal but also larvicidal[210] and powerful anticancer agents, as in the case of murine models of colon cancer (CT26), lung adenocarcinoma (3LL), melanoma (B16F10), and Ehrlich ascites carcinoma (EAC) cell lines.[209] As in the case of "green" AgNPs tested against cancer cells, these Ag_2ONPs were also specifically active against all the experimental malignant cells (both *in vitro* and *ex vivo*) but spared the normal ones, showing a certain degree of selectivity.

These important results evidence the potential of Ag_2ONPs as chemotherapeutic drugs for future use, which should be evaluated without hesitation.

4. Silver fact checking

Once the Chemist has gathered all the experimental evidences about silver efficacy in medicine, fact checking can start.

- "Silver can kill more than 650 different germs, viruses, bacteria, and fungi within 5-7 minutes". This statement was also found in the version "Silver can cure more than 650 diseases, pathogens and other conditions", which does not make much sense. Such a claim is evidently exaggerated, but has been propagated over the years without the support of any source or reference. The origin of this news is to be found in an article on silver sulfadiazine efficacy published in 1973,[212] where 657 isolates (i.e. pure strains of bacteria separated by a mixed bacterial culture) representing 22 bacterial species were inhibited by the silver drug. The 22 bacterial species included *P. aeruginosa*, *P. multiphilia*, *Klebsiella* sp., *Enterobacter* sp., *E. cloacae*, *E. coli*, *Proteus mirabilis*, *P. morganii*, *Serratia* sp., *Citrobacter* sp., *Herellea* sp., *S. aureus*, *S. epidermidis*, *Enterococcus* (group D *Streptococcus*).

- Silver is selective against "bad" bacteria while leaving the "good" ones unaffected. False. An impressive number of studies have reported silver, both in its metallic and cationic forms, to be

effective on almost all the bacterial species and strains tested for antimicrobial action, both Gram-positive and Gram-negative, to various degrees depending on the species, with slightly greater activity against Gram-negative bacteria.[213] Experimental studies suggest that concentrations of 60 ppm Ag^+ should be sufficient to control the majority of bacterial and fungal pathogens.[214]

- Silver is effective against fungal infections: true. Silver nanoparticles have displayed antifungal action especially on *Candida albicans*, in several studies,[215-217] whilst being scarcely toxic on human erythrocytes with low hemolytic effects. AgNPs have been evaluated for their antimycotic properties on dermal species such as *Candida glabrata, Candida parapsilosis, Candida krusei,* and *Trichophyton mentagrophytes* with good results.[174]

- Silver is an antiviral agent. True. The efficacy of silver nitrate against Herpes simplex virus at low concentration (30 μM or less) has been reported as early as 1976,[218] while the antiviral action of AgNPs on Herpes simplex and parainfluenza virus type 3 has only recently been described.[219] Moreover, AgNPs have shown activity against HIV-1, Tacaribe virus (TCRV), hepatitis B virus (HBV), recombinant respiratory syncytial virus (RSV), monkey pox virus, murine norovirus (MNV)-1, and influenza A/H1N1 virus.[27,146] To the best of our knowledge, there is no evidence that AgNPs could also be active against West Nile, Ebola and SARS viruses.

- Silver is an anticancer agent. True. As just seen above, a series of silver compounds ranging from AgNPs and Ag_2ONPs to Ag(I) complexes with NHC, phosphines, polypyridines, phenanthrolines, etc. display strong anticancer activity.

- Silver is able to heal gastrointestinal conditions (ulcer, diarrhea, stomach bug and colitis). True and false. There is evidence that nanocrystalline silver is able to treat ulcerative colitis, at least in rats,[220] but there is no sound proof that it can also treat other medical conditions associated with gastrointestinal problems, although silver could be effective against the pathogens which cause them.

- Silver can treat skin problems (acne, warts, dermatitis, eczema, psoriasis, seborrhea, hemorrhoids, lupus, and rash). True and false. Silver demonstrated to be effective against bacteria and inflammation processes, so these are the only skin conditions that could be relieved by this agent. Only silver nitrate

has been indicated in wart eradication with fair results.[65] To the best of our knowledge, there is no evidence that silver can heal autoimmune diseases: instead, there might be clues about silver induction of autoimmunity in rats.[221]

- Silver can treat eye infections. True. Silver nitrate has a long history in the treatment of ophthalmic infections. While AgNPs show low toxicity towards eye cells, they do not seem to be equally effective.[222]

- Silver can treat cystitis. True. Nanocrystalline silver has been reported to decrease bladder inflammation.[223]

- Silver can relieve allergies. True, at least in the case of contact allergies in murine models.[177]

- Silver can cure syphilis and gonorrhea. True. Silver arsphenammina has been used to cure syphilis while silver proteinates, such as Protargol and Argyrol, were employed against gonorrhea, at the beginning of 1900s and before the advent of antibiotics (*vide supra*).

- Silver is able to treat diabetes. True. There is recent evidence that "green" synthesized AgNPs can be effective against this metabolic disease.[224]

- Silver is effective against malaria. True. Both Ag(I) complexes and AgNPs have shown antiprotozoal activity, especially against malaria.[115]

- Silver can cure chronic arthritis. True. The efficacy of silver against this painful condition was probably tested for the first time in Vienna in 1928 with a colloidal silver preparation of Collargol[225,226] but it has also been recently confirmed.[227]

- Silver can treat appendicitis, diphtheria, pyorrhea, poliomyelitis, scarlet fever, tetanus, typhus and cough, catarrh, chronic fatigue syndrome, problems with the nervous and locomotor systems. False. Although it is clear that bacterial and inflammatory conditions could be relieved by silver action, as previously discussed, verification of most of these other claims is rather difficult since there is no precise information in the literature.

Conclusions

So far so good? Surely not. Although several silver formulations are prescribed and sold to treat certain medical conditions, most of the compounds described in this review, including those showing outstanding properties as antimicrobial or anticancer agents, are still in early stages of assessment, that is, *in vitro* studies, and may not make it to the clinical trials.

In fact, although silver salts, complexes and nanoformulations can be highly active *in vitro*, their *in vivo* efficacy could be enormously decreased or even completely abated, as a result of silver's strong interaction with cellular components, such as anions (chlorides, phosphates, sulfates, carbonates, sulfides, selenides), proteins (albumins, macroglobulins, etc.), thiol-containing species (glutathione metallothioneins) and other molecules that can reduce, bind and sequester the active Ag^+ ions, preventing them from reaching their cellular targets and accomplishing their therapeutic mission. A similar behavior is observed in silver nanoparticles, where the interaction of AgNPs with biological fluids leading to formation of AgNPs protein coronas, or corona components exchange taking place while the particle travels inside the cell, can have unexpected results *in vivo*, ultimately interfering with and affecting their biological activities (anticancer, antimicrobial, antifungal, etc.).

Moreover, the biodegradability and clearance pathways of silver nanoparticles should be studied and clarified, since long-term deposition of metallic nanoparticles in vital organs and tissues can cause severe toxicity. Hence biodegradability and clearance mechanisms need to be assessed before moving to clinical trials. While polymeric nanoparticles and micelles are rapidly degraded *in vivo* and easily eliminated, the degradation process of metallic nanoparticles is slow and may present clearance issues.[228-230] Biogenic metallic nanoparticles, however, are cleared from the body via urination, showing that phytochemically stabilized nanoparticles could have potential therapeutic and diagnostic applications.[231]

Furthermore, the contrasting evidence regarding silver (both ionic and nanometallic) toxicity should be solved once for all with studies involving more complex models in order to guarantee a safe use in therapeutic applications. At the very least, toxicity limits should be univocally assessed: cisplatin

is highly toxic but is successfully used in the medical practice. Thus, silver anticancer compounds do not have to be harmless at any cost, provided their toxicity limits are accurately evaluated and measured against possible drawbacks so that the risks do not outweigh the benefits. Different strategies can also be sought in order to decrease AgNPs's toxicity, for instance tuning the rate of silver ion release and using proper capping or coating on the nanoparticle surface.

Finally, strict quality controls and safety protocols should be established both in manufacturing silver compounds and nanoparticles, and in their potential therapeutic applications, in order to increase safety and efficacy.

A final remark concerns the practice of home-made and uncontrolled self-administration of colloidal silver, which can be dangerous for human health. Silver nanoparticulate suspensions can be pure in theory, but in practice they are most likely to be mixtures consisting of silver ions, nanoparticles, sub-nano sized particles and aggregated nanoparticles that are either nano-sized or greater. Moreover, the sources of silver in Do-It-Yourself preparations cannot always be controlled and certified, so that they may also contain dangerous metals as impurities that could pose serious health threats, from allergies to poisoning.

While colloidal silver is not known to have caused any death and some of its formulations are recognized as therapeutics, and although the World Health Organization still includes colloidal silver (produced by electrolysis of silver electrodes in water and water filters) in its list of water disinfection methods specified to provide safe drinking water in developing countries,[232] the FDA has concluded that the risk of using silver products exceeds any unsubstantiated benefits.[48] Although in 1996 the FDA proposed to ban over-the-counter products containing silver salts or colloidal silver, a Final Rule was issued in 1999 and became effective thereafter. The rule applies to any nonprescription colloidal silver or silver salt product claimed to be effective in preventing or treating any disease. It is still possible to sell silver products under the form of "dietary supplements" provided that no health claims are made for them. The FDA keeps issuing warnings to those companies that disregard the rules and make illegal therapeutic claims about colloidal silver products on their Web sites.

List of Abbreviations

Extracellular Polymeric Substances	EPS
N-Heterocyclic Carbenes	NHC
Nanocrystalline Silver	NCS
Nanosilver Particles	NPSs
Reactive Oxygen Species	ROS
Silver Nanoparticles	AgNPs
Silver Chloride Nanoparticles	AgClNPs
Silver Oxide Nanoparticles	Ag_2ONPs
Standard Reduction Potential	SRP
Viable But Not Culturable	VBNC

Ancillary Information

*Corresponding authors: Serenella Medici, sere@uniss.it, Massimiliano Peana, peana@uniss.it

Serenella Medici is an associate professor of Inorganic and Bioinorganic Chemistry at the Department of Chemistry and Pharmacy, University of Sassari, Sardinia, Italy. She teaches Inorganic and General Chemistry, Metals in Medicine, and Materials, Technologies and Conservation of Cultural Heritage. Her research interests focus on different aspects of the Transition Metals Chemistry, including synthesis, characterization and applications (e.g. catalysis and biomedical utilization) of metal complexes and metal nanoparticles, together with the study of metal-protein interactions and metal toxicology.

Massimiliano Peana is a researcher in Inorganic and Bioinorganic Chemistry at the Department of Chemistry and Pharmacy, University of Sassari, Sardinia, Italy, where he teaches Inorganic and General Chemistry, Organic Chemistry, and Sciences applied to Cultural Heritage. His current research interests are focused on metal interactions with peptides and proteins, molecular mechanisms of metal and metal nanoparticles related toxicity, carcinogenesis and neurodegeneration, chelating agents for toxic metals. His research in based on the use of several spectroscopic techniques, especially NMR, which was deepened during a four year's fellowship at CERM, Centre for Magnetic Resonance, University of Florence (2003-2007).

Valeria M. Nurchi is professor of Analytical Chemistry at the University of Cagliari, Italy. The majority of her works dealing with solution equilibria were mainly focused on (i) theoretical studies on the factors affecting the reliability of equilibrium parameters, (ii) computer programs for the calculation of complex formation constants, and (iii) studies on the interactions between metal ions and ligands of biomedical and environmental interest.

Maria Antonietta Zoroddu is full professor of General and Inorganic Chemistry at the University of Sassari, Department of Chemistry and Pharmacy, Italy.

Her major research interests include development of spectroscopic techniques such as Nuclear Magnetic Resonance and Electron Paramagnetic Resonance, Infrared, Ultraviolet and Visible Spectroscopy and their application to environmental and biological problems, study of structure and function of molecules and macromolecules, speciation of metal ions in biological systems and molecular mechanisms involved in the toxicity and carcinogenicity of metal ions.

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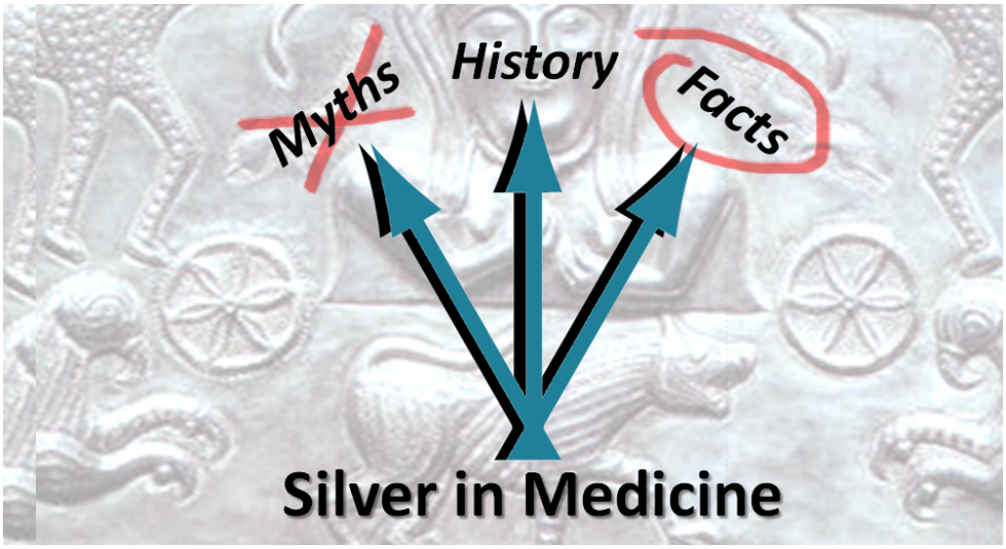

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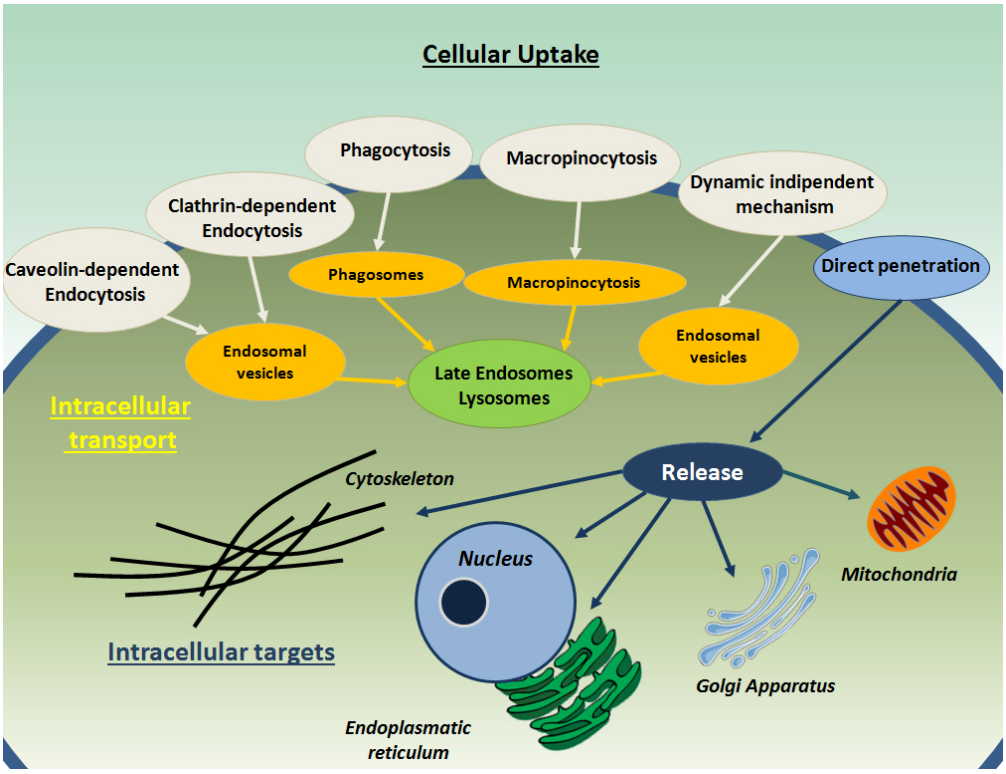

Figure 1. Cellular uptake, intracellular transport and targets of Silver Nanoparticles.

85x65mm (300 x 300 DPI)

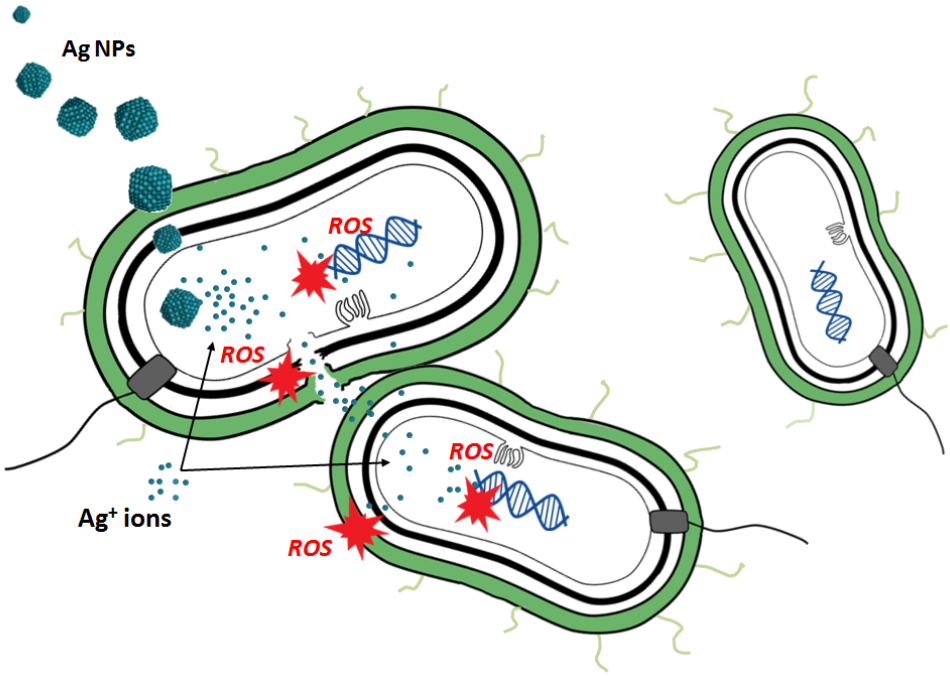

Figure 2. The zombie-effect of Silver-killed bacteria that become deadly to other bacteria. The silver ions that remain trapped inside them later cause the death of other bacteria.

50x35mm (500 x 500 DPI)

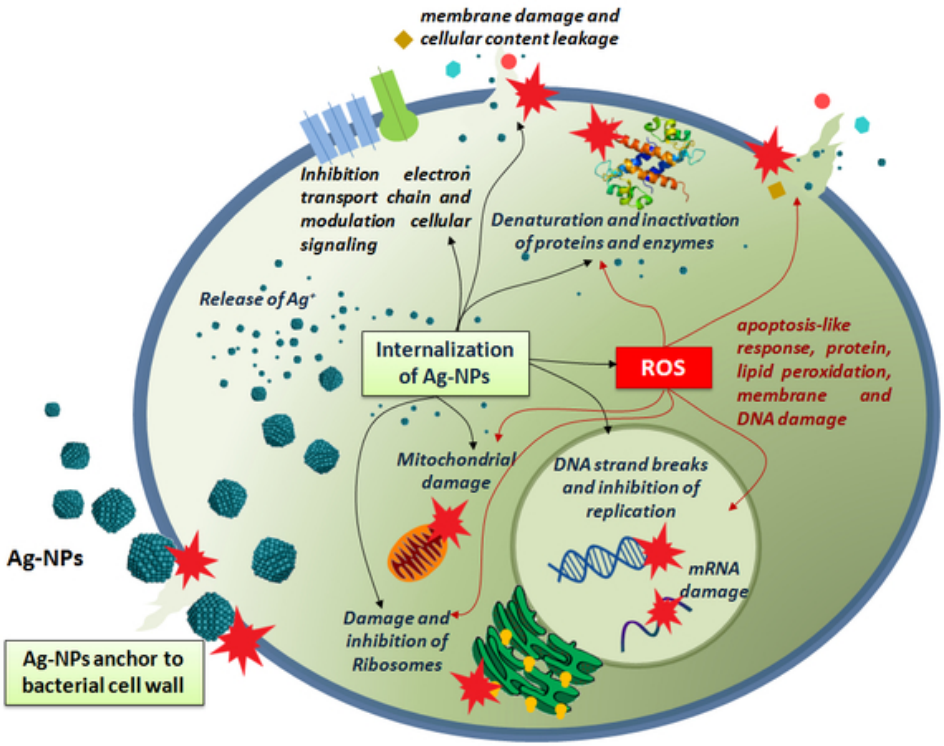

Figure 3. Antimicrobial routes of action of AgNPs: anchor to bacterial cell wall, internalization inside the cell and nucleus, release of Ag+ ions, cellular toxicity, ROS generation, membrane damage and modulation of cell signaling.

51x38mm (300 x 300 DPI)

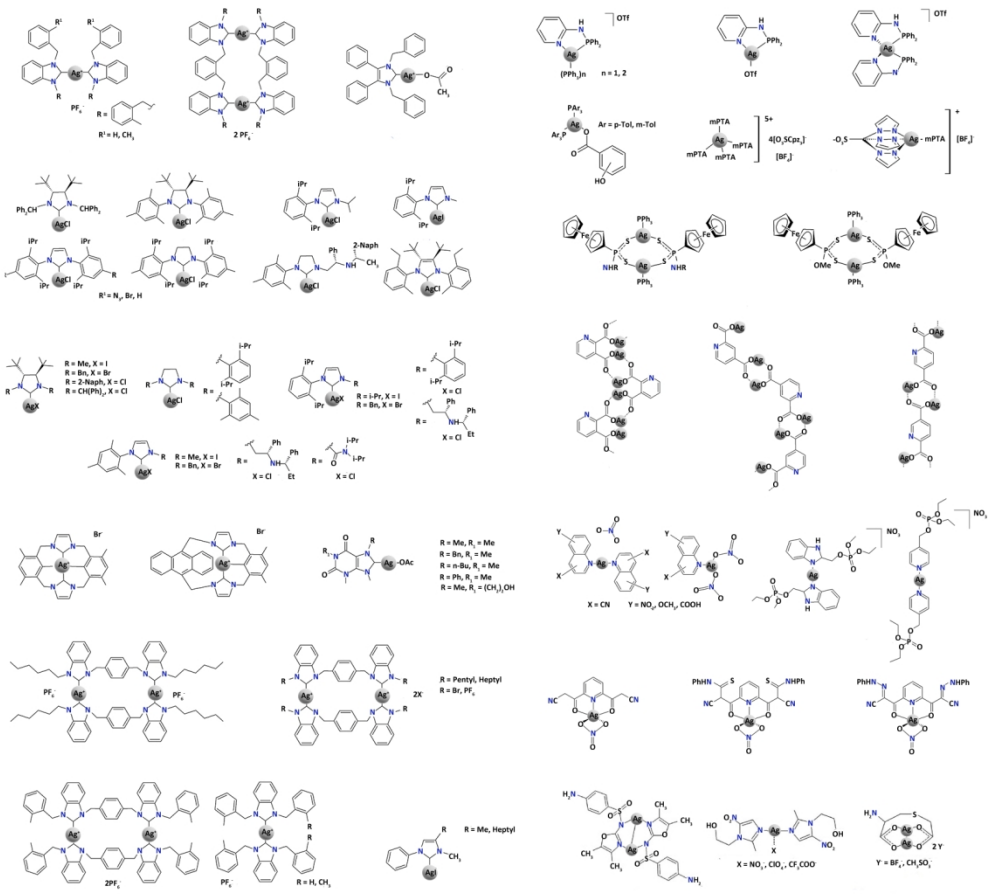

Figure 4. Examples of common Silver coordination compounds.

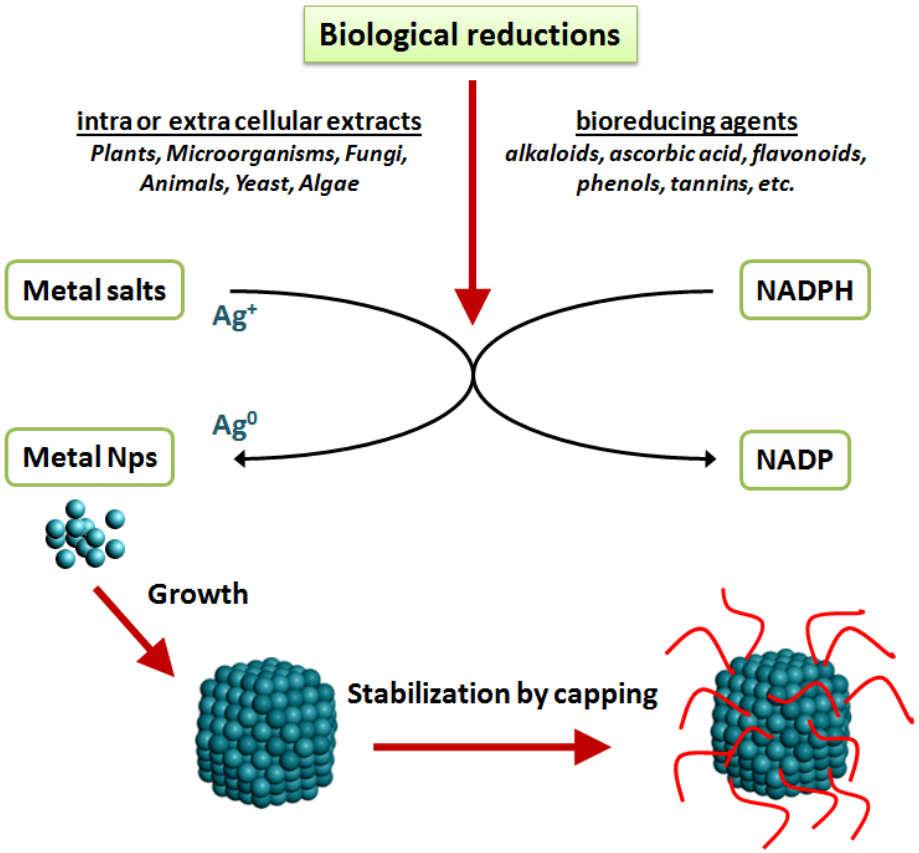

Figure 5. Mechanism of AgNPs biosynthesis.

68x60mm (300 x 300 DPI)

Tables

Table 1: Some silver compounds described in Squires' Companion to the BP (19th ed, 1916)

Name	Definition	Uses	% of Ag
Albargin	Gelatose silver or silver glutin	Bowel wash and treatment of gonorrhea	15
Argentamin	Silver phosphate in ethylene-diamine solution	Antiseptic astringent and disinfectant	10
Argonin L	A compound of silver nitrate and casein-soda	Treatment of gonorrheal ophthalmia and purulent ophthalmia	10
Argyrol	A compound of silver with a wheat protein	Wide range of indications especially in ophthalmic practice	30
Collargol	Colloidal silver used as a solution and in an ointment.	Ophthalmic indications	
Ichthargan	Silver ichthyolate	Infections of the genitourinary tract	30
Largin	Silver albuminate	Treatment of gonorrhea	11
Protargol	Silver protein	Treatment of chronic inflammation of the conjunctiva.	8

Table 2: Examples of organism-derived silver nanoparticles

Organism	Source Name	Size (nm)	metabolites involved in bioreduction	Pharmacological applications
Plant	*Acalypha indica*	20–30	Quercetin, plant pigment	Antibacterial
Plant	*Alternanthera sessilis*	40	Amine, carboxyl group	Antioxidant, antimicrobial
Plant	*Andrographis paniculata*	67–88	Alkaloids, flavonoids	Hepatocurative activity
Plant	*A. mexicana*	20–50	Protein,	Antimicrobial
Plant	*Artemisia nilagirica*	70–90	Secondary metabolites	Antimicrobial
Plant	*Boswellia serrata*	7–10	Protein, enzyme	Antibacterial
Plant	*Caria papaya*	15	Hydroxyl flavones, catechins	Antimicrobial
Plant	*Cassia fistula*	55–98	Hydroxyl group	Antihypoglycemic
Plant	*Cinnamon zeylanicum*	45	Water soluble organics	Antibacterial
Plant	*Citrullus colocynthis*	5–70	Polyphenols	Antioxidant, anticancer
Plant	*Citrus sinensis*	35	Water soluble compounds	Antibacterial
Plant	*Dillenia indica*	11–24	Biomolecules	Antibacterial
Plant	*Dioscorea bulbifera*	8–20	Diosgenin, ascorbic acid	Antimicrobial
Plant	*Euphorbia prostrata*	52	Protein, polyphenols	Antiplasmodial
Plant	*Gelsemium sempervirens*	112	Protein, amide, amine group	Cytotoxicity
Plant	*Lippia citriodora*	15–30	Isoverbascoside compound	Antimicrobial
Plant	*Mentha piperita*	90–150	Menthol	Antibacterial
Plant	*Mirabilis jalapa*	100	Polysaccharides	Antimicrobial
Plant	*H. canadensis*	113	Phenolics, protein	Cytotoxicity
Plant	*Iresine herbstii*	44–64	Biomolecules phenolic compound	Biological activities
Plant	*Melia azedarach*	78	Tannic acid, polyphenols	Cytotoxicity
Plant	*Tinospora cordifolia*	34	Phenolic compound	Antilarvicidal
Plant	*Trigonella-foenum graecum*	15–25	Flavonoids	Catalytic
Plant	*Withania somnifera*	5–40	Methyl 7-oxooctadecanoate	Antimicrobial
plant	*Alfalfa sprouts*	2-20		
plant	*Cinnamomum camphora*	55-80		
plant	*Azadirachta indica (Neem)*	50-100		
fungi	*Phoma sp. 3.2883*	71-74		
fungi	*Fusarium oxysporum*	5-15		Antibacterial and anticancer
fungi	*Verticillium*	13-37		
fungi	*Aspergillus fumigates*	5-25		
fungi	*Aspergillus flavus*	9		
fungi	*Trichoderma asperellum*	13-18		
fungi	*Phanerochaete chrysosporium*	50-200		
fungi	*Fusarium semitectum*	20-25		
bacterium	*Pseudomonas stutzeri*	200		Antibacterial
bacterium	*Streptomyces albidoflavus*	10-14		
bacterium	*Klebsiella pneumonia*	5-32		Antibacterial
bacterium	*Bacillus subtilis*	5-60		DNA-binding
Yeast	*Yeast MKY3*	2-5		
Algae	*Scenedesmus sp.*	15-20		Antimicrobial