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Silver bullets: a new lustre on an old antimicrobial agent

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Glossary:

Antibiotic: First coined in 1941 by Selman Waksman, the term is derived from the Greek “anti-life” or "opposing life, in this case of pertaining to the life of microbes.

Argyria: This is a rare, irreversible skin condition that tends to occur after an ingestion of silver salts or other preparations containing silver over a prolonged period of time.

Bactericide: A substance that kills bacteria directly.

Biofilms: Group of microorganisms growing together and then sticking to the host cells or surface accompanied by a matrix of extracellular material.

Colloid: A mixture of two or more dispersed insoluble particles.

Drug resistance: The reduction in effectiveness of an antimicrobial in curing infections due to a reduced susceptibility of the target microorganism. Resistance is predominantly a consequence of widespread overuse of antibiotics. This has led to a global threat to human health which is compounded by the scarcity of new antibiotics in the clinical pipeline.

Inflammation: A localized response elicited by endogenous danger signals, exogenous pathogen associated signals, injury or other destruction of cells, tissues, or organs. In the context of infection, an inflammatory response by a host serves to destroy the injurious agent, however chronic or extreme inflammation can be deleterious to the host.

MIC: Minimum inhibitory concentration, the lowest concentration of a substance that prevents the visible growth of bacteria.

ROS: Reactive oxygen species are chemically reactive substances containing oxygen.

Synergy: An effect produced by combining two agents wherein the result is greater than that observed for sum of the results elicited by the individual components.

Abstract

Silver was widely used in medicine to treat bacterial infections in the 19th and early 20th century, up until the discovery and development of the first modern antibiotics in the 1940s, which were markedly more effective. Since then, every new antibiotic introduced to the clinic has led to an associated development of drug resistance. Today, the threat of extensive bacterial resistance to antibiotics has reignited interest in alternative strategies to treat infectious diseases, with silver regaining well-deserved renewed attention. Silver ions are highly disruptive to bacterial integrity and biochemical function, with comparatively minimal toxicity to mammalian cells. This review focuses on the antimicrobial properties of silver and their use in synergistic combination therapy with traditional antibiotic drugs.

Keywords:

antimicrobial activity, drug-resistance, silver nanoparticles, synergism

1. The History of Medicinal Silver

Silver is one of the 'noble metals' with symbol Ag derived from the Latin word argentum. Argentina, a country of South America with an abundance of silver, was indeed named after this valuable mineral(Calvert P.A.R. 2017). The precious metal has been used in currencies, jewelry, decorative objects, kitchen utensils, and medicinally. Prior to the 21st century, silver was utilized for its antimicrobial properties in mainly domesticated forms such as needles, vessels, plates, cutleries, and even crude silver fillings (Figure 1).

The word 'silver' in modern day English is derived from the Anglo-Saxon word 'siolfur', denoting a shiny substance. The term "blue-blood" was used to describe members of upper-class society, and stems from a medical condition in which the skin of a person discolors to a bluish-grey tinge after a significant exposure of silver, first notated by Avicenna, who treated diseases using silver nitrate.(Alexander 2009) The phrase arose in the Middle Ages when only the upper social class could afford to use silver in their everyday utensils, such as silverwares and cutleries. Little did they know that the silver in these implements has a tendency to ionize into ions that easily permeate the skin.(Griffith, Simmons et al. 2015) Fortuitously this skin condition found favor amongst them when the bubonic plague struck, as "blue-bloods" had a higher chance of survival. This coincided with the scholarly discovery of the antimicrobial properties of silver.(Barillo and Marx 2014)

Figure 1: **Exemplary applications of silver related products along the course of human history**

In China during the Han Dynasty ca. 1500 BC fine acupuncture needles were crafted from silver.(Morones-Ramirez, Winkler et al. 2013) 300 years later (ca. 1300 BC), Phoenicians preserved liquids in clay jars lined with silver.(Cunliffe 2011) The Macedonians applied silver plates on wounds to prevent infections in ca. 800 BC.(Barillo and Marx 2014) Around 536 BC by Persian King Cyrus and around 335 BC by Alexander the Great, silver vessels were used to store drinking water during military campaigns. (Meurant 1994, Alexander 2009) Hippocrates (400 BC) used silver in powder form to treat ulcers and other infectious diseases.(Dai, Huang et al. 2010) The Romans introduced silver nitrate into the contemporary Roman pharmacopoeia ca. 69 BC.(Calvery, Lightbody et al. 1941) During the Middle Ages, wealthy Europeans used household cutlery and dinnerware made out of silver (500-1500 AD).(Cunliffe 2011) Around 920 AD, Avicenna (a Persian scholar and writer) introduced silver fillings to treat various diseases from bad breath to irregular heartbeats and even to purify blood.(Wadhera and Fung 2005) Paracelsus, a Renaissance alchemist, used silver nitrate salt as caustic to treat wounds in 1520. While around 1614, Angelo Sala (Italian physician and chemist) ingested silver nitrate solutions as laxative and for the treatment of brain infections.(Hill and Pillsbury 1939) A new treatment for vesico-vagina fistulas was reported by Dr. Marrion J. Sims, an American physician, in 1852. Dr. Albert Coombs Barnes, an American physician and Dr. Herman Hille, a German Chemist, patented Argyrol as an over-the-counter drug in 1950.(Hodson and Gillies 1985)

Silver as a medicinal component was almost forgotten after the discovery of modern day antibiotics. Antibiotics were initially considered as a wonder drug, but the potential for

bacteria to evade their effects by developing resistance was recognized by Fleming even before penicillin was widely used.(Davies and Davies 2010) Nowadays silver is being reconsidered as a potent antimicrobial agent with new inventions and new technologies derived from silver in various stages of development. In the modern era, the antimicrobial properties of silver have been exploited in a more sophisticated manner that is targeted to the treatment of diseases, using refined silver salts, colloidal silver, and especially silver nanoparticles.

A search of US patents from 2000-2017 show various commercialized fields of application of silver nanoparticles due to their antimicrobial properties. Innovative technologies utilizing silver for its antimicrobial properties that have been patented cover multiple areas, such as: domestic cleaning products enhanced with antimicrobial silver,(Miner and Eatough 25 Dec. 2007) antimicrobial surface coatings (potentially for food or medical devices),(Nabutovsky, Bornzin et al. 11 Feb. 2014, Hunt, Harris et al. 28 Jan. 2010), washable keyboards with an antimicrobial silver ion surface,(Whitchurch, Vaillancourt et al. 22 Oct. 2009) refrigerators with antimicrobial surfaces for better food preservation,(Kim 24 Aug. 2010) washing machines with colloidal silver,(Lee 21 Feb. 2011) wound dressings containing antimicrobial silver for scarring improvement,(Ma and Yu 9 Dec. 2008) antimicrobial food packaging,(Glenn, Vogt et al. 16 Aug. 2007, Schiffmann and Schlösser 29 Mar. 2011), silver odor eliminating air purifiers,(Bae, Lee et al. 8 May 2012, Kim and Kim 16 Aug. 2005) antimicrobial fabric embedded with silver nanoparticles(Tessier, Radu et al. 8 Oct. 2009, Rubinsky and Taylor 27 Aug. 2013) and water purification systems incorporating silver.(Harvey 8 Oct. 2013, King 09 Dec. 2013, Pradeep, Chaudhary et al. 24 Jul. 2014) Figure 2 demonstrates visually how the interest in the development and usage of silver as an antimicrobial agent has been steadily increasing over the last years (2000-2017). The graph shows a correlation between the number of published papers and patents. Within the published patents, a correlation between patents describing nanoparticles and those focused on antibacterial properties can be found. The number of patents about silver nanoparticles began substantially increasing after 2004, though in recent years patents mentioning 'silver' and 'antibacterial' have overtaken those mentioning 'silver nanoparticles'. In the years 2014-2016 the number of publications reporting antibacterial activity of silver was similar to the number of registered patents with the same terms.

Figure 2: **Innovative technologies utilizing silver due to antimicrobial properties**

Publications and patents that encompass the utilization of silver for antimicrobial activity published from 2000 to 2017. The trend chart was generated with search results obtained from SciFinder® (American Chemical Society) searching for the phrase 'antibacterial + silver'. This figure demonstrates how the development and usage of silver as antimicrobial agent has been steadily increasing. In 2000, less than two hundred patented applications were based on the concept, but by 2017 the number rose to almost 1500 patents incorporating the use of antibacterial silver products.

2. Forms of silver and synthesis of silver nanoparticles

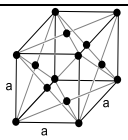
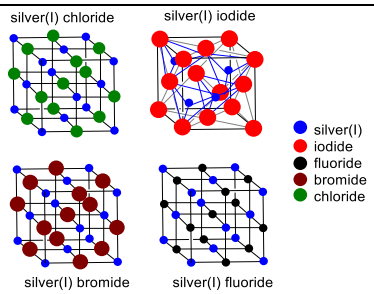
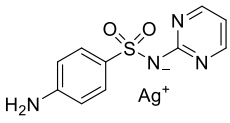
Over time, many have sought to use silver related products for therapeutic treatments, however the distinction between different forms of silver has been poorly addressed, and questions have arisen regarding the safety and efficacy of silver. Recent reviews summarize how certain forms of silver are superior to others as bactericides.(Mathur, Jha et al. 2017, Reshma, Syama et al. 2017) Table 1 outlines different forms of silver and their associated products that are used for medical and healthcare purposes. Silver nanoparticles by themselves cover distinct forms such as metallic nanoparticles and silver oxides (Ag_2O). For silver oxides and silver salts to be bactericidal, they first must disintegrate into silver ions. An enhancement of the antimicrobial activity of ionic silver has been described by using silver oxynitrate (Ag_7NO_{11}), where highly oxidized silver is stabilized with oxygen.(Kalan, Pepin et al. 2017) In this publication, the combination of non-toxic silver oxynitrate and commercially available wound dressings was reported to release silver ions and to disrupt biofilm formed from multidrug-resistant pathogens (blaNDM-1-positive *Klebsiella pneumoniae* and blaVIM-1-positive *Pseudomonas aeruginosa*).(Kalan, Pepin et al. 2017)

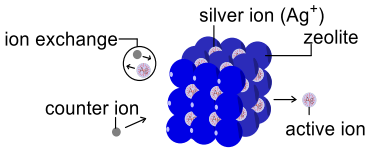
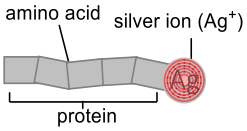
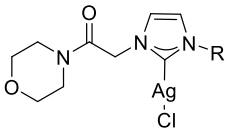
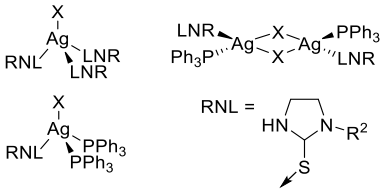
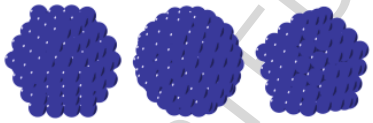
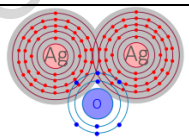
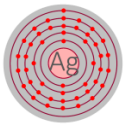
Silver diamine fluoride is a promising drug to treat or prevent the development of caries, evaluated in over 20 clinical studies, with the fluoride penetrating deeper into the tooth compared to other fluorides.(Fung, Duangthip et al. 2017, Mei, Nudelman et al. 2017, Burgess and Vaghela 2018, Horst 2018) As well as dental caries, silver diamine fluoride can relieve dentinal hypersensitivity. It is cleared by the U.S. FDA as a medicament and has been commercially available in several countries for many decades.(Fung, Duangthip et al. 2017, Mei, Nudelman et al. 2017)

There are various examples (see Table 1) where the combination of bioactive drug-loaded silver nanoparticles increases the efficacy of wound healing treatments(Berthet, Gauthier et al. 2017), for example by employing crystalline silver or silver sulfate.(Kee, Stockton et al. 2017) Silver sulfate can be employed as a component in wound dressings to help kill bacteria and speed up the healing process, and therefore it is used in commercial products sold by global companies.(Kee, Stockton et al. 2017) Cysteine-modified molybdenum disulfide infused with silver ions and then coated with a cationic polyelectrolyte could be an alternative antibacterial depot suitable for treating wound infections.(Cao, Ju et al. 2017) Other wound dressings rely on the combination of the antibacterial properties of metals with natural or synthetic polymers such as polysaccharides.(Naseri-Nosar and Ziora 2018) Examples are cotton cellulosic films impregnated with silver nanoparticles(Pannerselvam, Jothinathan et al. 2017), formulations of silver sulfadiazine and bacterial cellulose,(Shao, Liu et al. 2016), or the combination of silver sulfadiazine with poly (ethylene glycol) hydrogels to control infections in burnt wounds.(McMahon, Kennedy et al. 2016) The combination of silver nanoparticles with keratin leads to an material with antibacterial activity against wound bacteria,(Shanmugasundaram and Ramkumar 2018), while the combination with cellulose leads to an antifouling material(Adepu and Khandelwal 2018), and chitin-silver nanocomposites were active against bacteria and fungi.(Solairaj and Rameshthangam 2017)

Silver nanoparticles are quite often in wound healing design as antiseptics to accelerate the recovery processes. Hydrogels with sericin-, chitosan-capped silver nanoparticles are proposed for reduction of the bacterial infection risk during the healing progress.(Verma, Kanoujia et al. 2017) Silver complexes containing silver(1) have been tested for their antimicrobial activity, such as silver *N*-heterocyclic carbene complexes that are toxic to bacteria while being non-hazardous towards red blood cells at similar concentrations(Aher, Das et al. 2017). Silver imidazolidine-2-thiones complexes have also shown bactericidal activity against *K. pneumoniae*, *S. typhimurium*, *S. aureus* and MRSA with MICs up to 5 μg/mL, one yeast, *C albicans*. The dinuclear complexes were found to be more active than the mono-nuclear analogues.(Aulakh, Lobana et al. 2018)

Table 1: Systematic tabulation of major silver derived products used for its antimicrobial properties.

Chemical Structure		Description / Application	Ref.
Pure metallic silver			
Native silver (Ag^0)		• Biologically inert form, but eventually dissipates into silver ions • Mainly used in crafting of jewelry, cutleries, coinage, medical equipment	(Alexander 2009)
Silver salt compounds			
Silver halides (AgCl, AgBr, AgI, Ag_2F, AgF, AgF_2)		• Photosensitive • Commonly used in photographic films • Used in traditional clinical X-rays	(Aubert-Viard, Martin et al. 2015, Vosmanská, Kolářová et al. 2015)
Silver nitrate	$AgNO_3$	• Widely used in history to prevent and cure various diseases • Also, used as cauterizing agent	(Alexander 2009, Barbasz, Kreczmer et al. 2016)
Silver sulfate	Ag_2SO_4	• Is a major component in "Mepilex Border Ag", a silver wound dressing produced by global Swedish company MÖLNLYCKE • potent antimicrobial activity killing a wide range of bacteria within 30 minutes	(Kee, Stockton et al. 2017)
Silver complexes			
Silver diamine fluoride	$AgF(NH_3)_2$	• Silver diamine fluoride as a topical medicament for the treatment and prevention of dental caries and to relieve dentinal hypersensitivity • Known as Saforide, Advantage Arrest, Cariesop, Bioride, FluoroplatV, Riva Star • Commercially available in many countries including China, Japan, Germany, Nepal, Brazil, Argentina, New Zealand, Australia and others for many decades • Cleared for sale by the U.S. FDA as a class II medical device for the treatment of dentinal hypersensitivity	(Fung, Duangthip et al. 2017, Mei, Nudelman et al. 2017, Burgess and Vaghela 2018, Horst 2018)
Silver sulfadiazine ($C_{10}H_9AgN_4O_2S$)		• Largely formulated as ointments and creams for infection control in burnt wounds	(Shao, Liu et al. 2015, McMahon, Kennedy et al. 2016)

Silver zeolite		• Porous mesh of sodium aluminosilicate containing silver ions • Slow release of bactericidal silver ion. • Potential dental material	(Belkhair, Kinninmonth et al. 2015, Singh, Park et al. 2015)
Silver protein complex		• 8% silver with albumin(protargol®) • Used to stain carbohydrates and nerve tissues in microscopy • Initial treatment for gonorrhea in 1897	(Sacco, Sechi et al. 2016)
Silver-*N* - heterocyclic carbene complexes		• Screening of silver complexes incorporating morpholine against *S. aureus* (MIC up to 6.25 µM), *Salmonella enterica* (MIC 50 µM) • Non-hazardous towards red blood cells at concentrations below 1.56 µM	(Aher, Das et al. 2017)
Silver imidazolidine-2-thiones complexes		• Series of silver(I)-complexes studied against *S. aureus* (MRSA), *K. pneumoniae, Salmonella typhimurium, Candida albicans* • Low cellular toxicity with high cell viability	(Aulakh, Lobana et al. 2018)
Silver nanoparticles			
Metallic nanoparticles (Ag^0)		• Comes in few shapes such as rods, spheres, and dendrites in size of 1-50 nm • Has different production methods (refer to section 3) • Vast applications in pathogenic infection control • Acticoat, a Smith and Nephew product, as one example of nanocrystalline silver, increasing rate of healing in wounds and burns by decreasing inflammation, maintaining moist, decreasing wound surface and reducing frequent dressing changes	(Shenashen, El-Safty et al. 2014, Franci, Falanga et al. 2015, Le Ouay and Stellacci 2015, Kee, Stockton et al. 2017)
Silver oxide nanoparticles (Ag_2O)		• Usually found with silver colloids • Used widely to coat surfaces which need antimicrobial properties	(Karunakaran, Jagathambal et al. 2016)
Monoatomic silver ion (Ag^+)		• Easily forms bonds and complexes which inactivate the bactericidal action by oxidative stress and cell wall perforations, microbe metabolism turbulence, and DNA disruption have also been observed • All forms of silver that disintegrate into this form proven to be bactericidal	(Jung, Koo et al. 2008)

2.1. Silver nanoparticles – their chemical and physical synthesis pathways

The advantage of nanoparticles in general is their high surface to volume ratio which allows strong interactions at therapeutic sites and their small size facilitating penetration through biological membranes. Silver nanoparticles consist of a range of nanostructures and crystallization forms. Different synthesis methods are employed to prepare silver nanoparticles, leading to variations in specific nanoparticle morphology and accompanying physical and chemical properties, such as conductivity, thermal and melting point.(Chaloupka, Malam et al. 2010, Shenashen, El-Safty et al. 2014, Wu, Yang et al. 2018) The morphology of crystal formation can be controlled based on the distance of crystal formation from the thermodynamic equilibrium.(Le Ouay and Stellacci 2015)

In this review, recently published synthesis procedures are described with an emphasis on their application. A widely used method to synthesize nanoparticles is chemical reduction of silver ions, employing chemical reducing agents such as sodium borohydride, citrate, ascorbate or hydrogen. Table 2a summarizes recently reported methods of silver nanoparticle synthesis with an emphasis on their application.

Silver nanoparticles synthesized by chemical reduction with sodium borohydride have been combined with a variety of other agents. Adenosine triphosphate coated silver nanoparticles have activity both against bacteria and cancer cells(Datta, Chatterjee et al. 2017), while cellulose fibers functionalized with silver nanoparticles and stabilized by dendrimers improve biocidal potency in textile products(Kebede, Imae et al. 2017). The functionalization of silver nanoparticles with vancomycin results in an enhancement of antimicrobial activity(Kaur, Goyal et al. 2017). Other antimicrobial materials were obtained by reducing silver nitrate with sodium borohydride in the presence of other additives, such as silver nanoparticles stabilized by sodium dodecyl sulfate(Schwass, Lyons et al. 2018), by cationic ligands(Dai, Chen et al. 2017), or by chitosan(dos Santos Junior, Targino et al. 2017). The products are capable of inhibiting biofilm formation and suggested to be used for the treatment of caries. Silver nanoparticles with keratin(Shanmugasundaram and Ramkumar 2018), cellulose(Adepu and Khandelwal 2018) and chitin(Solairaj and Rameshthangam 2017) were prepared using chemical reduction method with sodium borohydride (keratin and cellulose) or trisodium citrate (chitin) as reductants.

Radiation can be also used for the reduction of silver salts to nanoparticles with, for example, gamma irradiation to form bioactive nanogels for the development of antimicrobial surfaces(Anjum, Patra et al. 2016) or by preparing nanosilver functionalized glass through photoreduction to prevent the development of oral biofilms(Paiva, Fidalgo et al. 2017). Furthermore, silver nitrate was reduced by electrical energy in the presence of polyethylene glycol and the morphology of the formed nanoparticles was controlled under various reaction conditions.(Nasretdinova, Fazleeva et al. 2015) Microwave energy can also reduce silver nitrate to induce silver nanocrystal formation.(Shenashen, El-Safty et al. 2014) Silver nanoparticles could be obtained by microwave sintering, with improved antibacterial activity depending on the temperature.(Shi, Zhou et al. 2017) In another study, silver nitrate reduced

by argon glow discharge was incorporated into functionalized cotton fabrics with an antimicrobial activity.(Li, Meng et al. 2017)

A thermal reduction method can be applied to embed silver nanoparticles on different materials by heating the reaction mixtures of silver salts and additives. Nanocomposites were obtained with varying properties: nanocomposites of graphene oxide and silver nanoparticles (heated at 60°C) were utilized to eliminate bacterial biofilms(Wang, Han et al. 2018), activated carbon fibers (self-assembled at 98°C, followed by carbonization) showed an antimicrobial activity(Yan, Xu et al. 2017), nanocomposites of silver and TiO_2 (heated at 100°C, then calcined at 500°C; heated again at 180°C) exhibited a synergistic antimicrobial activity against *Escherichia coli*(Wang, Chen et al. 2017, Ye, Cheng et al. 2017) and *Staphylococcus aureus*(Ye, Cheng et al. 2017). The combination of silver nanoparticles with zeolite (heated at 90°C) resulted in an antibacterial material active against methicillin-resistant *S. aureus*(Chen, Popovich et al. 2017), while functionalization with chitosan (hydrothermal method employing 140°C)(Biao, Tan et al. 2017) or the preparation of composite membranes with silver loaded sepiolite (heated at 150°C)(Díez, Santiago-Morales et al. 2017) were accompanied with an increased antibacterial activity. Nanostructured silver vanadates were proposed as an antimicrobial agent against a range of pathogenic bacteria or for dental applications.(de Melo Monteiro, Dias Holtz et al. 2018)

Table 2a: Recently published methods for the synthesis of silver nanoparticles with emphasis on their application.

	General overview of the method	Advantage	Targeted application	Ref.
Chemical Reduction with $NaBH_4$	ATP (adenosine triphosphate) coated silver nanoparticles were prepared in phosphate buffer (pH 7.4) at 37°C with $NaBH_4$ as a reducing agent	Novel method of nucleotide metallization via interaction of silver nanoparticles with adenosine triphosphate	Treatment of human cancer, excellent bactericidal activity over both Gram-positive and Gram-negative bacilli	(Datta, Chatterjee et al. 2017)
	Silver nanoparticles prepared from an aqueous $AgNO_3$ solution mixed with PAMAM, $NaBH_4$ was added to the reaction mixture to obtain dendrimer silver nanoparticles which were bound on viscose rayon fiber	Good biocidal activity against *E. coli*	The immobilization of functional metal nanoparticles protected by dendrimer on cellulose fibers as textile products with smart functions to minimize sick building syndromes	(Kebede, Imae et al. 2017)
	Nanoparticles were synthesized using trisodium citrate and PVP as capping agents, $NaBH_4$ as reducing agent and $AgNO_3$ as precursor.	Functionalized with a glycopeptide antibiotic	Model system for the development of new bactericides	(Kaur, Goyal et al. 2017)
	Reduction of $AgNO_3$ with $NaBH_4$ in the presence of sodium dodecyl sulfate	Inhibition of biofilm formation by *Streptococcus spp.* and *Enterococcus faecalis in vitro*	Disinfection of carious dentine	(Schwass, Lyons et al. 2018)
	Reduction of $AgNO_3$ with $NaBH_4$, sodium citrate and addition of $NH_3{\cdot}H_2O$ and NaOH in water	Size-tuneable nanoparticles with highly uniform morphologies and narrow size distributions	Antibacterial activity, against *E. coli* and *S. aureus*	(Wu, Yang et al. 2018)
	Reduction of $AgNO_3$ in the presence of trisodium citrate	Antibacterial material by combining silver nanoparticles and keratin	Antibacterial activity against burn wound bacteria	(Shanmugasundaram and Ramkumar 2018)
	Mixing of $AgNO_3$ with the described polymer and addition of $NaBH_4$	Activity against antibiotic-resistant Gram-negative, Gram–positive bacteria and bacterial biofilms	Therapy of pathogenic bacterial infections	(Dai, Chen et al. 2017)
	The silver-bacterial cellulose (BC) composites were synthesized by mixing $AgNO_3$ with $NaBH_4$ and BC	Bacterial cellulose-based antifouling material	Protection of food from microbial spoilage	(Adepu and Khandelwal 2018)
	$AgNO_3$ was reduced with $NaBH_4$, sodium citrate and PVP as a stabilizing agent	High antimicrobial activity and low environmental and cytotoxic effects	Alternative antimicrobial substance against fish pathogens, novel therapeutics in aquacultures	(Shaalan, El-Mahdy et al. 2017)
	Addition of $AgNO_3$ to a chitosan solution in acetic acid, followed by addition of $NaBH_4$	Size and shape of the nanoparticles had no influence on the antimicrobial properties against *Streptococcus mutans*	Treatment of caries	(dos Santos Junior, Targino et al. 2017)
Reduction using radiation	Nanosilver embedded polyacrylamide nanogels were synthesized using $AgNO_3$ in a gamma irradiation process	Bioactive nanogels with antimicrobial potency against *E. coli* and *S. aureus*	Development of antimicrobial surfaces in surgical devices and synthetic implants	(Anjum, Patra et al. 2016)
	Photoreduction of silver nanoparticles in a polyacrylate solution	Production of nanosilver glass ionomer cements with an antibacterial effect by diffusion	Dental application by preventing the development of oral biofilms	(Paiva, Fidalgo et al. 2017)

Thermal reduction method	Silver nanoparticles were synthesized on graphene oxide nanosheets in the presence of poly (vinyl pyrrolidone) at 60°C	Nanocomposites of graphene oxide with silver chloride/silver with high visible light absorption and antibacterial properties	Elimination of bacterial biofilms under visible light irradiation for applications as wound dressing or water purification	(Wang, Han et al. 2018)
	HBPAA-capped silver nanoparticles were self-assembled on viscose fibers at 98°C, followed by peroxidation and carbonization	Activated carbon fibers functionalized with silver nanohair with durable antibacterial activity	Activated carbon fibers with antibacterial activity	(Yan, Xu et al. 2017)
	Ag/titanium glycolate particles were heated at 100°C, then calcined to 500°C	Macroporous Ag/TiO_2 composite foams with synergistic antimicrobial activity against *E. coli*	Material with bactericidal activity	(Wang, Chen et al. 2017)
	Synthesis of Ag/carbon spheres using $AgNO_3$, CTAB, glucose at a temperature of 180°C. The TiO_2/Ag composite was obtained employing the spheres in the presence of tetrabutyl titanate at 180°C	Spherical titanium dioxide/silver composite showing synergistic effects against *E. coli* and *S. aureus* with a bacteriostatic rate up to 99%	Durable antimicrobial coating for application in bacterial sensitive locations	(Ye, Cheng et al. 2017)
	The glass preservative was mixed with silver oxide and other materials and melted at 950°C	Inorganic UV filter incorporating TiO_2, Ag and ZnO	Integration into polymers, UV screen textiles or coatings	(de Lucas-Gil, Rubio-Marcos et al. 2016)
	Preparation of thin films by multi-magnetron sputtering	Coatings based on titanium and silver with a biocidal effect	Nanocrystalline films as antimicrobial coatings	(Wojcieszak, Mazur et al. 2017)
	Combination of zeolite suspension with dissolved $AgNO_3$ at 90°C	Silver-ion-exchanged nanostructured zeolite with antimicrobial activity against methicillin-resistant *S. aureus*	Antibacterial material based on nanostructured zeolite	(Chen, Popovich et al. 2017)
	Synthesis of silver colloid using a hydrothermal method (140°C) with chitosan both as reductant and stabilizer	Chitosan-functionalized silver nanoparticles with bactericidal effect against bacteria and fungus	Potential bactericidal agent	(Biao, Tan et al. 2017)
	Nanoparticles obtained at 150°C	Membranes with metal loaded sepiolite showing antibacterial activity	Composite membranes with silver as antibacterial activity	(Díez, Santiago-Morales et al. 2017)
	Nanosilver oxide synthesis with PVA, PVP, chitosan and $AgNO_3$ and calcination at 600°C	Suppression of gastric inflammation and inhibition of oxidative stress	Antiulcerogenic potential of nanosilver oxide for the prevention of gastric ulcers	(Salem, Wahba et al. 2017)
	Reaction of $AgNO_3$ with carboxymethyl functionalized cellulose in the presence of citric acid at 95°C	Combination of non-toxicity with antibacterial activity against *S. aureus, E. coli, P, aeruginosa*	Nanocomposites incorporated in hydrogel membranes for skin wound dressings or skin repair substitutes	(Capanema, Mansur et al. 2018)
Other reduction methods	Reduction of $AgNO_3$ with polyaniline-coated or polypyrrole-coated fabrics	Coated cotton fabric with silver nanoparticles with good antibacterial activity against *S. aureus* and *E. coli*	These cotton fabrics coated with conducting polymers could be applicable for the potential electrostimulation	(Maráková, Humpolíček et al. 2017)
	$Ca(NO_3)_2$ hydrate, $AgNO_3$ and $(NH_4)_2HPO_4$ reacted together followed by conventional and microwave sintering	Higher antimicrobial activity of particles sintered by microwave	Improved antibacterial activity with increase of temperature	(Shi, Zhou et al. 2017)
	$AgNO_3$ reduced by chitosan and sericin (reducing and stabilizing polymer) to produce chitosan-	Accelerated wound healing with improved patient compliance	Silver-based antiseptics, reduction of the risk of bacterial infection during the healing process	(Verma, Kanoujia et al. 2017)

	and sericin-capped silver nanoparticle loaded hydrogels			
	Formation of silver nanoparticles on cotton fabrics by reducing $AgNO_3$ with argon glow discharge as the electron source	Fabrication of silver nanoparticles on cotton without using any chemical reducing agent	Functionalized fabrics with high antimicrobial activity for wearable devices	(Li, Meng et al. 2017)
	Reduction of $AgNO_3$ with trisodium citrate, followed by addition to chitin nanoparticles	Chitin-silver nanocomposite with an enhanced antimicrobial effect on bacteria and fungi	Usage as a biocompatible larvicidal material	(Solairaj and Rameshthangam 2017)
	Mixing $AgNO_3$, chitosan-L-glutamic acid and hyaluronic acid	Antimicrobial activity against *E. coli* and *S. aureus* and promotion of wound healing	Spongy composites used as antibacterial wound dressings	(Lu, Lu et al. 2017)

2.2. Silver nanoparticles – "Green" synthesis methods

"Green" synthesis methods consider environmental issues, such as selection of less toxic solvents, reducing agents, and stabilizers. Over the past decade, numerous publications have reported variety of protocols with silver nanoparticles synthesized by a biological method rather than a chemical one. A comprehensive summary of all known eco-friendly methods is out of the scope for this review, but we summarize a variety of approaches in Table 2b. „Green" synthesis routes make use of plant, wood and fruit extracts, bacteria, fungi or other sources. Depending on the synthesis method, the size and morphology of the produced silver nanoparticles vary, as do their biological properties.

Generally, silver nitrate is used as a precursor for the bio-reduction processes with biological sources used as reducing agents. Some plants contain caffeine and theophylline that are known to act as stabilizers for silver ion formations. Numerous attempts have generated nanoparticles from many different types of plant components including green tea, moringa and henna leaves, clove and cinnamon bark extract, germanium plants, chestnut trees isolates, and mangosteen fruits.(Shenashen, El-Safty et al. 2014, Rupiasih, Aher et al. 2015, Rajput, Raghuvanshi et al. 2017) The use of plant material to hasten the reduction process was successful in terms of high yields and rapid syntheses, however the downside is that parameters such as the size and morphology of the nanoparticle products are hard to control.(Shenashen, El-Safty et al. 2014, Ahmed, Ahmad et al. 2016)

Wood extracts can be used for the biosynthesis of nanoparticles, for example a *Eucalyptus* extract employed as the reductant for $AgNO_3$.(Shivakumar, Nagashree et al. 2017) These synthesized nanoparticles possesed an antimicrobial activity against both bacteria and fungus. Bark extracts of *Guazuma ulmifolia*(Karthika, Arumugam et al. 2017) and *Prosopis juliflora(Arya, Kumari et al. 2017)* reduced $AgNO_3$ at ambient temperature resulting in nanoparticles showing activity against several pathogens. Other plant extracts reducing $AgNO_3$ in aqueous solution include leaf extracts from *Erythrina suberosa* rich in glycosides, flavonoids and phenolic compounds, and capable of reduction at room temperature.(Mohanta, Panda et al. 2017) Aqueous leaves extracts from *Artemisia haussknechtii*(Alavi and Karimi 2017), *Bryophyllum pinnatum*(Tareq, Fayzunnesa et al. 2017), *Protium serratum*(Mohanta, Panda et al. 2017), *Thymbra spicate*(Erci, Cakir-Koc et al. 2017) and *Angelicae Pubescentis Radix* root (Markus, Wang et al. 2017) all yielded silver nanoparticles with antimicrobial activity against food, multi-drug resistant, agriculture or plant pathogens.

Fruit extracts are also described to act both as the reducing and stabilizing agent for the synthesis of silver nanoparticles. Reaction of *Actinidia deliciosa* and $AgNO_3$ at 80°C yielded silver nanoparticles with antibacterial and anticancer activity.(Naraginti and Li 2017) The silver nanoparticles synthesized with aqueous extracts of *Prunus cerasifera* showed anti-bactericidal action against several strains.(Jaffri and Ahmad 2017)

Fungi can be employed as silver nanoparticle secreting hosts, with an advantage of high yielding production of highly stable silver nanoparticles. The main mechanism works by trapping Ag^+ in the fungal cell where multiple reduction enzymes can act on the ions.(Shenashen, El-Safty et al. 2014) Mycosynthesis has been performed using cell free filtrates of *Trichoderma viride*(Kumari, Shukla et al. 2017), *Penicillium chrysogenum*(Sheet, Sathishkumar et al. 2017), *Chaetomium globosum*(Singh, Kumar et al. 2018) or *Aspergillus niger* plus *Fusarium semitectum*(Madakka, Jayaraju et al. 2018) to yield silver nanoparticles with activity against various bacteria or cancer cells. The supernatant of several bacterial strains has also been employed to reduce $AgNO_3$, including *Bacillus amyloliquefaciens* and *Bacillus subtillis*(Fouad, Hongjie et al. 2017, Ghiuță, Cristea et al. 2017), *Streptomyces xinghaiensis*(Wypij, Czarnecka et al. 2018) or *Novosphingobium*(Du, Singh et al. 2017). The products showed antimicrobial activity against several pathogens. Similarly, nanoparticles were produced by silver resistant bacteria possessing nicotinamide adenine dinucleotide phosphate hydride (NADPH) and NADPH-dependent enzymes such as nitrate reductase, including *K. pneumoniae, E. coli, E. cloacae, A*er*omonas sp.SH10*, *Corynebacterium sp.SH09* and *Bacillus licheniformis*.(Prabhu and Poulose 2012, Shenashen, El-Safty et al. 2014)

Other strategies and further reducing methods for the synthesis of silver nanoparticles include the use of mushroom extracts of *Ganoderma applamatum* as a reductant for $AgNO_3$ at room temperature, forming silver nanoparticles with antibacterial activity(Mohanta, Singdevsachan et al. 2016). Natural amino acids can act both as reducing and capping agent for nanoparticle synthesis.(Kumar, Bansal et al. 2018) In another approach, the combination of light irradiation and rice straw biomass successfully produced silver nanoparticles with antibacterial activity.(Li, Ma et al. 2017) Silver nanoflowers were synthesied in the presence of poly-dopamine copper phosphate to yield selective antibacterial agents.(Zhang, Peltier et al. 2017)

"Green" synthesized silver nanoparticles have found similar application to those synthesized chemically or physically. They have been used in antimicrobial, anti-inflammation, or anticancer medical fields as wound healing accelerators, in antiseptic clothing or for other biomedical purposes. They are recognized as very attractive components for the food and agriculture sectors, and in the pharmaceutical industry, thanks to their very low toxicity and environmentally-friendly production.

Table 2b: Recently published methods for the green synthesis of silver nanoparticles with emphasis on their application.

	General overview of the method	Advantage	Targeted application	Ref.
Biosynthesis using wood extracts	Synthesis using pre-hydrolysed liquor (generated from pulp and paper industries) of *Eucalyptus* wood as reductant and $AgNO_3$	Biosynthesized silver nanoparticles with antimicrobial activity against bacteria (*P, aeruginosa, S. aureus, E. coli*) and fungus	Growth inhibitors for biomedical applications	(Shivakumar, Nagashree et al. 2017)
	Preparation with a bark extract of *Guazuma ulmifolia* and $AgNO_3$ at room temperature	Catalytic activity as reducing agent, able to bind to CT-DNA (groove-binding mode), antimicrobial activity on microbial pathogens	Bark- synthesized nanoparticles as antimicrobial, antifungal, anticancer agents	(Karthika, Arumugam et al. 2017)
	Reduction of $AgNO_3$ using *Prosopis juliflora* bark extract at room temperature	Antibacterial activity against *E. coli* and *P, aeruginosa,* anticancer activity against A549 cell line	Antimicrobial, anticancer activity	(Arya, Kumari et al. 2017)
Biosynthesis using plant extracts	Aqueous leaf extracts of *Erythrina suberosa* containing glycosides, flavonoids, phenolic compounds with $AgNO_3$ at room temperature	Antimicrobial activity against pathogenic bacteria and fungi	Antimicrobial, anticancer agent and wound healing application	(Mohanta, Panda et al. 2017)
	$AgNO_3$ stirred with aqueous *Artemisia haussknechtii* leaves extract at room temperature	Antibacterial activity against multi-drug resistant bacteria (*S. aureus*, *S. epidermidis*, *Serratia marcescens* and *E. coli*)	Antibacterial activity	(Alavi and Karimi 2017)
	Silver nanoparticle preparation using *Bryophyllum pinnatum* leaves extract and $AgNO_3$	Activity against food (*E. coli*) and agriculture (*Bacillus megaterium*) pathogens	Application in the food (food safety) and agricultural sector (agricultural production)	(Tareq, Fayzunnesa et al. 2017)
	Reduction of $AgNO_3$ with aqueous extract of roots of *Angelicae Pubescentis Radix*	Antimicrobial activity against *E. coli, S. aureus, P, aeruginosa, Salmonella enterica*	Potential application as drug carriers to inflammation-sites or medical imaging	(Markus, Wang et al. 2017)
	Aqueous extract of *Dionaea muscipula, D. binate, D. spatulata, D. indica* tissue and PVP were used to reduce $AgNO_3$ at 70°C	Antibacterial activity against human and plant pathogens	Antimicrobial properties	(Banasiuk, Krychowiak et al. 2017)
	Reaction between $AgNO_3$ and leaves extract of *Protium serratum*	Antibacterial activity against food borne pathogens (*P, aeruginosa, E. coli, B. subtilis*)	Antibacterial agent in food packaging and preservation	(Mohanta, Panda et al. 2017)
	Reaction between aqueous leavesextracts of *Thymbra spicate* and $AgNO_3$ at room temperature	Antibacterial activity against Gram-negative and Gram-positive bacteria	Controlling biological activity of the silver nanoparticles	(Erci, Cakir-Koc et al. 2017)
Biosynthesis using fruit extracts	*Actinidia deliciosa* fruit extract (reducing and stabilizing agent) added to $AgNO_3$ and reacted at 80°C	Antimicrobial activity (*P, aeruginosa*), anticancer activity (tested against human colon cancer HCT116 cells)	Field of biomedicine, water treatment, nanobiotechnology	(Naraginti and Li 2017)
	Reduction of $AgNO_3$ by chemical reduction ($NaBH_4$) using an aqueous extract of pomegranate peel (stabilizer)	Antibacterial activity against *E. coli*	Antibacterial activity	(Nasiriboroumand, Montazer et al. 2018)
	Synthesis of silver nanoparticles using aqueous fruit extract *Prunus cerasifera* (angiospermic plant) and $AgNO_3$	Antimicrobial activity against *Xanthomonas citri, P, syringae, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus terreus, Penicillium chrysogenum, Fusarium solani, Lasiodiplodia theobromae*	Application as nanobactericides and nanofungicides	(Jaffri and Ahmad 2017)
M yc	Aqueous solution of $AgNO_3$ reduced and modified using	Antimicrobial activity against *Shigella sonnei, P, aeruginosa* and *S.*	Pharmaceuticals and agriculture	(Kumari, Shukla et al. 2017)

	secondary metabolites of cell free filtrate of *Trichoderma viride* at 28°C	*aureus* with enhanced internalization of the synthesized nanoparticles compared to particles which have been stabilized by citrate	pharmaceutical industry	
	Synthesis using culture filtrates of *Penicillium chrysogenum* and $AgNO_3$ at 25°C	Antimicrobial activity against *E. coli*, *P, aeruginosa, S. aureus, Enterococcus faecalis* and cytotoxicity against cancer cells	Field of nanotechnology, astrobiology	(Sheet, Sathishkumar et al. 2017)
	Aqueous $AgNO_3$ and cell free filtrate of endophytic *Chaetomium globosum*	Antimicrobial activity against *S. aureus, K. pneumoniae*	Bactericidal activity for drug development of nanoantibiotics	(Singh, Kumar et al. 2018)
	Reduction of $AgNO_3$ with cell free filtrates of fungi *Aspergillus niger* and *Fusarium semitectum*	Antimicrobial activity against *E. coli, S. aureus, P, aeruginosa*	Antimicrobial agents for medical purposes	(Madakka, Jayaraju et al. 2018)
Biosynthesis using bacteria	Reduction of $AgNO_3$ by *Bacillus amyloliquefaciens, B. subtillis* (aqueous enzymatic extracts from cell free supernatant as reducing agent) at 33°C	Antimicrobial activity against *E. coli, P, aeruginosa, Salmonella, S. aureus, Streptococcus pyogenes, Candida albicans*	Exploiting antibacterial effect, potential in the optical domain (for example colour tuning by nanoparticle incorporation) or biosensor assays	(Ghiuță, Cristea et al. 2017)
	Cell free supernatant of bacteria strains (*B. amyloliquefaciens, B. subtilis*) was used to reduce $AgNO_3$	Toxic against the larvae and pupae *Culex Pipiens pallens*	Combination of bacteria silver nanoparticle with antibacterial activity to control vector mosquito and bacteria	(Fouad, Hongjie et al. 2017)
	Silver nanoparticles obtained using supernatant of *Streptomyces xinghaiensis* and $AgNO_3$ at room temperature	Antimicrobial activity against *E. coli, P, aeruginosa, S. aureus, B. subtilis, Candida albicans, Malassezia furfur*	Potential for application in nanomedicine	(Wypij, Czarnecka et al. 2018)
	$AgNO_3$ reduced with supernatant of bacterial strain *Novosphingobium* at 25°C	Antimicrobial activity against *S. aureus, Candida tropicalis, P, aeruginosa, E. coli, Vibrio parahaemolytics, Candida albicans, Salmonella enterica, B. subtilis, Bacillus aureus*	Antimicrobial agent against multidrug-resistant bacteria	(Du, Singh et al. 2017)
Biosynthesis using other methods	Mushroom extract from *Ganoderma applanatum* was reacted with $AgNO_3$ at room temperature	Antibacterial activity against *E. coli, B. subtilis, S. epidermidis, Vibrio cholerae, S. aureus, Shigella flexneri*	Use in medical devices, as antimicrobial agent, production of nanomedicines	(Mohanta, Singdevsachan et al. 2016)
	$AgNO_3$ was reduced by rice straw biomass at room temperature using light irradiation	Antimicrobial activity against *E. coli, P, aeruginosa, B. subtilis, S. aureus*	Silver nanoparticles as an adjuvant drug treatment against bacteria	(Li, Ma et al. 2017)
	Combination of silver nanoparticles ($AgNO_3$) and Fe_3O_4 nanoparticles ($FeCl_3$ hydrate and $FeSO_4$ hydrate) using starch as stabilizer and capping agent to produce nanocomposites	Antibacterial activity evaluated in a wound biofilm model	Treatment of chronic wound infections	(Ghaseminezhad, Shojaosadati et al. 2017)
	Silver nanoparticles were prepared from $AgNO_3$and curcumin (reducing and capping agent)	Antibacterial and antibiofilm activity while being less toxic to human keratinocytes	Wound dressing agent	(Jaiswal and Mishra 2018)
	Synthesis of silver nanoparticles with natural α-amino acids (reducing and capping agent) and $AgNO_3$	Antimicrobial activity against *E. coli, S. aureus, K. pneumoniae, P, aeruginosa*	Antimicrobial agents against drug-resistant strains	(Kumar, Bansal et al. 2018)

	Reaction of PD/copper phosphate nanoflowers with $AgNO_3$	No need of external toxic reductants, selective toxicity towards bacterial cells	Treatment of infections, sterilization of surfaces	(Zhang, Peltier et al. 2017)

3. Silver's mechanism of action

The precise mechanism by which silver induces bacterial cell death is still not fully understood.(Durán, Durán et al. 2016) Multiple studies have concluded that silver ions can disrupt more than one cellular process leading to its bactericidal action(Durán, Durán et al. 2016, Mathur, Jha et al. 2017, Reshma, Syama et al. 2017) (a schematic presentation of one mechanism is presented as a component of Figure 3). Silver nanoparticles can dissociate to biologically active silver ions (Ag^+) that have been shown to bind to bacterial cells walls via a "Trojan Horse" mechanism that then draws more silver ions into the cell.(Singh, Shedbalkar et al. 2015) The anchoring and penetration of silver nanoparticles to the bacterial cell wall results in structural changes, changing cell wall permeability. This activity, together with the formation of free radicals, damages the cell membrane.(Mathur, Jha et al. 2017) Bacterial cell walls are comprised of peptidoglycan, a polymer composed of sugar and amino acid residues. The sugar molecules, namely *β*-(1,4) linked *N*-acetylglucosamine and *N*-acetylmuramic acid, are attached to a peptide chain made of three to five amino acid which cross-links to form a mesh around the cytoplasm. Studies have proposed that silver nanoparticles and silver ions can interact with this layer to form pits by destroying the cross-linking bonds.(Agnihotri, Mukherji et al. 2014) Pits formed in the cell wall will cause internal cell content to leak out to the surrounding media and cause cell disintegration.(Jung, Koo et al. 2008, Rai, Yadav et al. 2009, Morones-Ramirez, Winkler et al. 2013, Franci, Falanga et al. 2015)

Another proposed mechanism of action is that the presence of silver ions triggers increases production of hydroxyl radicals which disrupt cellular regulations. The overproduction of reactive oxygen species can be triggered when the metabolic pathways, which are driven by Fenton chemistry, are disrupted by silver ions.(Morones-Ramirez, Winkler et al. 2013) It has been shown that reactive oxygen species (ROS) induced by silver nanoparticles can lead to cell apoptosis.(Quinteros, Aristizábal et al. 2016, Saini, Saha et al. 2016, Patil and Kim 2017) ROS production increased under visible light irradiation by graphitic carbon nitride nanosheets entrenched with silver nanoparticles resulted in an enhancement of antibacterial efficacy.(Bing, Chen et al. 2015) Again, the bacterial cell membrane is rendered unstable and more permeable to the extracellular environments.(Kumari, Shukla et al. 2017) The interactions of silver ions with thiol groups of cellular proteins is reported to lead to poor protein folding.(Russell and Hugo 1994) When these misfolded proteins are localized on the cellular membrane, the overall membrane structure will be further weakened and more permeable.

Studies propose that silver ions bind to the functional groups of key enzymes, halting cell division as well as cell metabolism.(Jung, Koo et al. 2008) Complexes of membrane targeting cationic ligands and silver nanoparticles could disrupt the membrane structure of bacteria (*S. aureus, P, aeruginosa*) resulting in an inhibition of intracellular enzyme activity.(Dai, Chen et al. 2017) It has also been proposed that bacteria exposed to silver ions over a long term basis end up with disrupted nucleic acid sequences.(Mijnendonckx, Leys et al. 2013) Silver ions have high tendencies to form stable complexes with nucleic acid preventing

transcription and stopping bacterial activity at the genetic level. The exact pathways have not yet been identified.(Franci, Falanga et al. 2015)

In summary, when bacteria cells are exposed to silver, the bacterial cell wall is punctured and degenerated, causing the cytoplasm to leak. Upon degradation of the bacterial cell wall, the bacteria become more susceptible to further influx of silver ions which causes disruption of cellular protein production and metabolic dysfunction. Cell growth and division is ultimately affected due to the silver ions' ability to cause genetic disruption and enzymatic inhibition.(Durán, Durán et al. 2016, Dai, Chen et al. 2017, Reshma, Syama et al. 2017) The activity of silver nanomaterials is dependent both on the size and the structure (nanoparticles, nanorods, nanofibers or nanomats).(Lu, Ye et al. 2014, Vimbela, Ngo et al. 2017)

Figure 3: **Silver ions mode of action and ways for the introduction of silver into the human body**

Entry methods and the effect of silver in the human body are summarized in this figure. Furthermore, a schematic model of the destructive forces of silver ions towards microbes is presented in the left upper corner. Silver ions can inhibit the growth of the microbe by perforating and weakening the cell wall of organism (1), by disrupting metabolic pathways (2) or by stopping the cell replication through attaching to DNA (3).(Jung, Koo et al. 2008, Morones-Ramirez, Winkler et al. 2013) Entry routes include: Skin: topical application of silver containing medication such as silver wound dressing, contact with silver particles, or elements. Particles smaller than 10 nm can pass through the pores of the tough outer layer (stratum corneum). Particles around 7-20 nm may penetrate through sweat and pilosebaceous glands. Particles around 20-200 nm will not penetrate the skin but remain at the follicle opening and they can enter systemic distribution through subcutaneous lymphatic system.(Chen and Schluesener 2008) Respiratory system: Inhalation of airborne silver particles. Particles first settle on the moist outer surface of the alveoli and can reside there for up to 7 days.(Chen and Schluesener 2008) Gastrointestinal system: Direct consumption of elemental silver or nanoparticles in the form of supplements and medication. Particles may reside on the sub-mucosal region of the intestine and be absorbed into the lymphatic or the capillary system.(Chen and Schluesener 2008, Eckhardt, Brunetto et al. 2013) Female urogenital: silver impregnated sanitary napkins for prevention of odor and infections.(Brandtzaeg 1997) Brain: silver nanoparticles and colloidal formulations in the blood stream circulate to the blood-brain barriers.(Eckhardt, Brunetto et al. 2013)

4. Silver and its effects on human health

Unlike copper, iron, and zinc, which are essential trace metals, silver does not naturally occurr in the human body. Figure 3 presents potential exposure routes allowing entry of silver into the body. Silver poisoning has been reported in individuals exposed to silver over a long period, with the severity of toxicity in general linked to the dosage.(Eckhardt, Brunetto et al. 2013) The toxicity of nanosilver can cause ill effects on humans as well as on the environment. Symptoms of silver exposure include a blueish discoloration of the skin, called argyria, or discoloration of the eye, called argyrosis. Exposure to soluble silver complexes can cause liver and/or kidney damage with the toxicity of silver affected by a number of factors, such as particle size, shape, surface or capping agents.(Mathur, Jha et al. 2017)

Toxicity studies on seven-week-old male rats with three daily oral treatments of 3.6 mg/kg silver nanoparticles for 14 days have shown that silver nanoparticles can lead to toxic effects on the host and the host gut microbiome.(Javurek, Suresh et al. 2017) The exposure of *Sprague-Dawley* male rats to different shapes of silver nanoparticles (highly dispersed, uniform nanocubes with a diameter of 50 nm, an average size of 84 nm and nanospheres with an edge length of 45 nm, average size of 93 nm) showed an impact on the gut microbiota\ and on some rat behaviors, but induced no significant histopathological changes in the gastrointestinal system and brain.(Javurek, Suresh et al. 2017) The authors concluded that the short-term and low dose exposure (two weeks) of silver nanoparticles to male rats led to both behavioral changes (anxiety-like, possibly stereotypical and increased repetitive behavior) and to gut microbiome changes, with the latter activity due to the nanoparticle antimicrobial activity affecting the bacteria comprising the gut microbiome.(Javurek, Suresh et al. 2017) Other rat experiements have shown an effect on neurotransmitters after exposure to silver nanoparticles (2.25 mg/kg), accompanied by changes in noradrenaline and dopamine levels in the brain.(Hadrup and Lam 2014) An autoimmune condition was found in mouse strains after addition of 0.5 g/L of silver nitrate in the drinking water.(Havarinasab, Pollard et al. 2009) The oral administration of silver nanoparticles (12 nm in size, 300 mg/kg/d) to female rats resulted in inflammation, apoptosis and degenerated follicles.(Ema, Okuda et al. 2017)

Silver may be stored, metabolized or excreted in the human body. Upon oral exposure, silver was deposited as particles in tissues such as the skin epidermis, glomeruli or intestines.(Hadrup and Lam 2014) Nanotoxicity could be induced by the unwanted distribution of nanoparticles to non-target tissue.(Mathur, Jha et al. 2017) In most cases of argyria affecting the skin, silver compounds are metabolized and silver residues are deposited in the skin. Ultrafine nanoparticles can normally be taken up by any organ and circulated throughout the body, resulting in systemic effects.(Chen and Schluesener 2008) Autopsies of patients who were identified as undertaking silver therapy have generally found that silver was not associated with their death, which was usually attributed to a progression of their underlying illness. For example, a 71-year-old male, taking silver combinations with an anti-androgen to suppress prostatic cancer, died 5.5 months after the consumption of

silver. His autopsy showed a brain with Alzheimer's type 2 and an intact nervous system.(Lansdown 2007) In another case both a 60-year-old man and an 18-year-old man developed localized argyria after being exposed to silver nitrate in wound dressings. There were no traces of neurological or behavioral changes, nor signs of accumulation in the brain. The most adverse case appears to be that of a woman (age not given) who had died from an extreme systemic shock and circulatory failure after being given a 7% silver nitrate solution to induce abortion. Autopsy showed that silver was present evenly over her entire body, including her brain, and her tissues were extremely congested.(Lansdown 2007)

Regardless of several reported adverse reactions, most studies have concluded that silver causes no direct or significant harm leading to death. It seems that systemic and localized argyria is the only major concern of using silver. An argyria reversing treatment has been developed for localized argyria pigments via treatment with a 1064 nm Q-switched Nd:YAG laser.(Griffith, Simmons et al. 2015) The mechanism of removal has yet to be fully understood, however it is believed to be somewhat similar to the removal of tattoo ink pigments where tattoo particles absorb the photons and photo-acoustically explode and are subsequently engulfed by circulating macrophage to be drained through the lymphatic system.(Griffith, Simmons et al. 2015)

In order to reduce toxicity, some strategies have suggested binding silver to sulfur molecules or enveloping it in biological membrane. However these approaches pose a challenge as altered silver nanoparticles are likely to be less effective at releasing bioactive Ag^+ ions.(Hatipoglu, Keleştemur et al. 2015) To minimize adverse effects such as allergies, rashes, itches, and swelling associated with the use of classical chemically synthesized particles, patient-friendly strategies free of "chemical" contamination have been suggested as a healthier option. The potential of photosynthesized silver nanoparticles for wound dressings were reported in an *in vivo* study in rats, where cotton fabrics incorporated with silver nanoparticles proved to increase wound healing activity compared to a silver-free commercial burn heal ointment named "Burn Heal" (CIPLA Ltd., India) of unknown content.(Pannerselvam, Jothinathan et al. 2017) Moreover, the anti-inflammatory activity of aqueous suspensions of silver nanoparticles was tested in animal model to alleviate colitis in mice as promising agents for the treatment of inflammatory bowel diseases.(Siczek, Zatorski et al. 2016) In another study, the hospital strain *P, aeruginosa,* resistant to many antibiotics, was sensitive to commercially available silver nanoparticles (10 nm) at concentrations of 0.156 μg/mL, which in cytotoxicity tests were shown to be safe for mammalian cell lines at concentrations of up to 2.5 μg/mL.(Salomoni, Léo et al. 2017)

As already mentioned in section 2, dental caries are treated with silver diamine fluoride cleared by the U.S. FDA as a medicament and commercially available in several countries (China, Japan, Germany, Nepal, Brazil, Argentina, New Zealand and Australia) for many decades.(Fung, Duangthip et al. 2017, Mei, Nudelman et al. 2017)

5. Silver versus other metals

Silver is not the only metallic element that possesses antimicrobial properties. Metals with bactericidal properties against Gram-positive and Gram-negative bacteria include gold, copper, zinc, titanium, nickel, magnesium, ruthenium and others.(Grass, Rensing et al. 2011, Xu and Imlay 2012, Dizaj, Lotfipour et al. 2014, Vimbela, Ngo et al. 2017, Mital and Ziora 2018) Most metals that are non-essential co-factors can cause harmful toxic effects in humans, even at the lowest concentration. Essential metals including chromium, copper, iron, magnesium, manganese, molybdenum, potassium, selenium, zinc, sodium, cobalt, lithium, nickel, silicon, and vanadium are metals which take part in biological processes in the human body such as, for example, in the regulation of cells. Essential metals must be consumed at an appropriate amount, too little and bodily function may suffer, while having too much will cause toxic side effects. An example can be seen in the human intake of zinc: when taken in slight excess (even by 3-10 mg) it results in copper levels in the system to drop which in turn leads to other complications.(Yasuyuki, Kunihiro et al. 2010) Moreover, experiments with mice fed with zinc for five weeks revealed dietary zinc to alter the gut microbiota, with increased levels resulting in intensified severity of *Clostridium difficile* infection.(Zackular, Moore et al. 2016) Other metals are classified to be completely toxic to humans, such as lead, tin, mercury, and tellurium.(Yasuyuki, Kunihiro et al. 2010, Lemire, Harrison et al. 2013)

Gold is more inert to oxidation and degradation than silver and is regarded to be safer in human biological systems.(Boda, Broda et al. 2015) Gold is a non-essential metal that will only display toxicity if administered in a relatively high concentration or in complexes with hazardous materials. Unlike silver, which has the tendency to cause argyria and other related poisoning, gold is known to be far more biocompatible.(Martin, LiLow et al. 2015) Gold has been widely used, especially in the field of carrier selection for drug and genetic therapies. This is mainly due to the benefits of gold nanoparticle, which can be generated with tailored morphology.(Martin, LiLow et al. 2015, Vimbela, Ngo et al. 2017) Gold has also been assessed for its antimicrobial properties. However, the mechanism in which gold acts as an antimicrobial agent differs from silver. Gold nanoparticles are known to reduce cellular ATP by inhibiting ATPase.(Boda, Broda et al. 2015) Also, gold has been observed to prevent protein synthesis by inhibiting the binding of tRNA to the ribosomal subunits.(Schröfel, Kratošová et al. 2014, Vimbela, Ngo et al. 2017) Some killing mechanisms are common with silver, such as interacting with peptidoglycan and preventing its synthesis.(Tiwari, Vig et al. 2011) In direct comparison, minimum inhibitory concentration (MIC) tests showed a higher activity of silver nanoparticles (MIC of 128 μg/mL for gold and 8 μg/mL for silver against Gram-negative bacteria *E. coli*, and MIC of 512 μg/mL for gold and 32 μg/mL for silver against Gram-positive bacteria *S. aureus*).(Ahmad, Wani et al. 2013) Another study demonstrated MIC values of cefaclor being reduced while complexed with gold nanoparticles against *S. aureus* and *E. coli*.(Rai, Prabhune et al. 2010) MIC values against *Streptococcus mutans* were stated as 4.9 μg/mL for silver and a higher value was found for gold (197 μg/mL).(Hernández-Sierra, Ruiz et al. 2008)

Copper is frequently used in household hardware and has long been recognized for its antimicrobial properties. In the agricultural sector, copper sulfate mixtures have been used to prevent fungal growth on agricultural produce such as grape vines, potato, peach trees, and apples. Even today, copper salts are widely applied as a preventive measure against infection outbreaks on food crops.(Lemire, Harrison et al. 2013) The over dosage of copper, an essential metal to the human body, has been known to cause toxic effects on the bone marrow and the liver. Regardless of side effects, copper can also be a versatile infection reducing agent with the cellular damage induced by reactions between released copper ions and the bacteria membrane.(Vimbela, Ngo et al. 2017) Over the years, bacteria biofilms have been observed to selectively avoid growing on copper surface such as copper door knobs and copper wires. Copper ions can also complex with bacterial nucleotides which retard repair mechanisms and bring cellular processes to a halt.(Martin, LiLow et al. 2015, Hoseinzadeh, Makhdoumi et al. 2017) The toxicity of copper nanomaterials was found to be size-dependent with smaller particles possessing a higher toxicity.(Vimbela, Ngo et al. 2017) Composites of copper and zinc loaded montmorillonites revealed a synergistic antibacterial activity against *E. coli*, and *S. aureus* with correlation to the specific surface area.(Jiao, Lin et al. 2017) Comparison of the efficacy of metals against bacteria strains *P, aeruginosa*, *S. aureus* and *E. coli* revealed copper to be more effective for the prevention of biofilm attachment, eradication of biofilms and prevention of planktonic cell growth than silver.(Gugala, Lemire et al. 2017) In this study, copper and titanium were found as the most effective metals against biofilm formation of all strains.(Gugala, Lemire et al. 2017)

Zinc is well known for its antimicrobial properties as it is often used in the production of cosmetic and skin care products (sold as an ingredient in skin care products to minimize acne), both as a preservative and as an antimicrobial.(Sirelkhatim, Mahmud et al. 2015, Padmavathy and Vijayaraghavan 2016) In some cases, zinc is taken in oral formulated tablets as supplements for those who have zinc deficiencies,(Gupta, Mahajan et al. 2014) and has been marketed to prevent colds.(Bauer 2015) Antimicrobial action is possible regardless of whether it is present as zinc salts, zinc-carbomers, zinc-complexes, or zinc oxides, as long as it ionizes into zinc ions.(Pasquet, Chevalier et al. 2015) Similar to silver nanoparticles, when zinc nanoparticles release bioactive ions, they destabilize the bacterial cell membrane and cell wall via generation of ROS.(Padmavathy and Vijayaraghavan 2016, Saini, Saha et al. 2016, Patil and Kim 2017) With bulk zinc oxide approved by the FDA as a 'generally recognized as safe' (GRAS) substance, zinc oxide nanoparticles are considered as biocompatible and suitable candidates for chemotherapeutic agents, drug carrier systems for loading and transporting drugs or antimicrobial agents.(Mishra, Mishra et al. 2017) Interestingly, a combination of silver and zinc oxide nanoparticles revealed antibacterial activity against *Mycobacterium tuberculosis* with no cytotoxic effects on macrophages revealing superior properties than each of the silver or zinc oxide nanoparticles alone.(Jafari, Mosavi et al. 2016)

Overall, the potential of metal ions as anti-microbial agents lies in their ability to supplement the limited existing options to fight multidrug-resistant and biofilm-related

infections.(Gugala, Lemire et al. 2017) The bactericidal activity of antimicrobial peptides has been enhanced by complexing metal-binding sites of the peptide antibiotics with transition metals.(Jeżowska-Bojczuk and Stokowa-Sołtys 2017) Moreover, synergistic effects against microbes were found for formulations of several metals mixed in combinatorial treatments.(Garza-Cervantes, Chávez-Reyes et al. 2017) Mixing of silver with nickel, cadmium, copper increased their antimicrobial activity significantly against *E. coli* while mixing with copper, zinc, cobalt enhanced the activity against *B. subtilis*. One explanation for the observed synergy relies on the increased cell permeability of these formulations.(Garza-Cervantes, Chávez-Reyes et al. 2017)

6. Synergistic effects of silver nanoparticles and antibiotics

In the search for solutions to overcome bacterial drug resistance(Butler, Blaskovich et al. 2016, Crofts, Gasparrini et al. 2017), one option is the combination of antibiotics with antibacterial metals such as silver(Nagy, Harrison et al. 2011), copper or gold. Studies combining silver nanoparticles with 'defeated' antibiotics have been initiated over the last ten years, with results successfully showing that silver can revitalize antibiotics to treat resistant bacteria. However, because the mode of action of antibiotics is diverse, with, some targeting the microbe cell wall synthesis and others affecting DNA/RNA or protein synthesis, synergistic effects aren't observed in all complexes. The first evidence that vancomycin, normally only a Gram-positive antibiotic, could also act on Gram-negative bacteria was found for its combinations with silver, not only enhancing the activity against bacteria but also offering a new mode of action.(Morones-Ramirez, Winkler et al. 2013)

Other approaches involving the combination of different antibacterial agents with either silver nanoparticles or silver nitrate have been reported in recent years. For example, the combination of silver nanoparticles and visible blue light was reported to enhance antimicrobial activity.(Akram, El-Tayeb et al. 2016, El Din, El-Tayeb et al. 2016) A triple combination of silver nanoparticles, blue light and antibiotics (amoxicillin or vancomycin) resulted in synergistic effects especially against methicillin resistant *S. aureus* (MRSA) isolates.(Akram, El-Tayeb et al. 2016) Improvement in activity of up to three orders of magnitude were reported for the combination of methylene blue and silver nitrate compared to the antibacterial agent alone, against a range of species (*Enterobacter cloacae*, *K. pneumoniae*, *P, aeruginosa*, *E. coli* and *Serratia marcescens*).(Li, Chen et al. 2016)

Table 3 presents various combinations of antibiotics with different forms of silver, with the antibiotics classified according to their structural class.

6.1. β-Lactam antibiotics and silver nanoparticles

The β-lactams in Table 3 are divided in penicillin, cephalosporin, and carbapenem subclasses incorporating the most successful and widely used antibiotics, yet those most threatened by resistance. The lactam antibiotics work by binding irreversibly to DD-transpeptidases that

facilitate the final crucial step of transpeptidation during peptidoglycan synthesis. Inhibition prevents interlinking between the subunits of the peptidoglycan layer and thus stops cell wall synthesis.(Fisher, Meroueh et al. 2005)

Resistance against β-lactam antibiotics emerged shortly after they were commercially launched in 1945.(Brown, Butterworth et al. 1976) One of the most common resistance mechanisms of bacteria involves the production of β-lactamases. These enzymes break down the β-lactam ring of the antibiotic that is crucial for its activity. Combining a β-lactam antibiotic such as amoxicillin with a β-lactamase inhibitor such as clavulanic acid can abrogate this resistance.(Leonard, Bonomo et al. 2013) Another resistance method is through the bacteria's alterations of DD-transpeptidases, observed in MRSA and penicillin-resistant *Streptococcus pneumoniae*.(Utsui and Yokota 1985) Methylation or glycosylation prevents the antibiotic from binding effectively to its binding site. The two resistance mechanisms mentioned above can be countered by simply linking β-lactam antibiotics to silver nanoparticles.

Increased activity could be observed for some complexes, with either additive or synergistic effects. It is difficult to compare results presented in the literature from different studies as authors report the observed interaction in a variety of ways, using different types of assays (e.g. broth microdilution vs agar zone of inhibition) against different bacterial strains. The results can be described as % increase, percentage, or FICI (Fractional Inhibitory Concentration)(Möhler, Kolmar et al. 2017), which cannot be directly compared. Keeping in mind that the difference in nanoparticles size and morphology can also affects the final activity, it is difficult to analyze the findings other than to present them.

The % increase was either obtained from the presented publications or calculated with data presented in the respective article. The equation was % increase = (x-y)/y where x is the inhibition zone for the antibiotic in combination with silver nanoparticles and y is the antibiotic alone. FICI values were calculated with the following equation (FICI values ≤0.5 indicate synergistic effects):

$$FICI = \frac{MIC_{antibiotic\ in\ combination}}{MIC_{antibiotic\ alone}} + \frac{MIC_{silver\ nanoparticles\ in\ combination}}{MIC_{silver\ nanoparticle\ alone}}$$

The activity of ampicillin, penicillin and amoxicillin was enhanced against a broad range of strains as summarized in Table 3. Strong synergistic effects were observed in combination with silver nanoparticles at concentrations of 2.5 mg/mL accompanied with no cytotoxic effect on mammalian cells.(Panáček, Smékalová et al. 2015) in the size of 28 nm against *E. coli*, *S. aureus*, *P, aeruginosa* showing a decreased MIC against the tested bacteria. Silver nanoparticles added to penicillin resulted in a-four-fold increase in activity against penicillin-resistant *S. pneumonia*, (Ghosh, Patil et al. 2011) though another study reported a much more moderate 12% increase in killing methicillin-resistant *S. aureus* by adding silver nanoparticles to methicillin (Devi and Joshi 2012).

Penicillin, ampicillin and feropenem combined with silver nanoparticles exhibited a significant increased activity against the bacteria strain *K. pneumoniae*.(Ghosh, Patil et al. 2011) The combination of nanocomposites of silver, titanium-nanotubes and penicillin were synergistic against *E. coli* (FICI of 0.38).(Xu, Cheng et al. 2017) Ampicillin with silver nanoparticles showed synergistic effects against five bacteria strains with FICI values ranging from 0.38 to 0.5.(Hwang, Hwang et al. 2012) Silver nanoparticles synthesized through a bacteria assisted procedure (with *S. xinghaiensis* OF1 strain) combined with ampicillin were synergistic against *E. coli*, *K. pneumoniae*, *S. aureus* and *B. subtilis* (FICI of 0.12).(Wypij, Czarnecka et al. 2018) Another significant improvement was observed for piperacillin complexed with silver nanoparticles against *A. baumannii* and *P, koreensis*.(Ghosh, Patil et al. 2011) Silver nanoparticles resulted in more potent agents against *S. aureus* when combined with penicillin (MIC: 0.06 μg L^{-1})(Panáček, Smékalová et al. 2015) and oxacillin (MIC: 0.48 μg L^{-1})(Panáček, Smékalová et al. 2015), and against *E. coli* with ampicillin.(Hwang, Hwang et al. 2012, Panáček, Smékalová et al. 2015) Synergistic activity with cefazolin and cephalothin was dependent on the size of the nanoparticle (Hari, Thomas et al. 2014) The performance of cefotaxime and ceftazidime was also improved when complexed with silver nanoparticles, with synergistic effects reported against *E. coli*, *K. pneumoniae* and *P, aeruginosa*.(Gurunathan 2015, Panáček, Smékalová et al. 2015, Panáček, Smékalová et al. 2016) Complexes with meropenem even showed an increased activity against resistant bacteria compared to susceptible strains.(Panáček, Smékalová et al. 2016)

6.2. Aminoglycoside, glycopeptide and ansamycine class antibiotics and silver nanoparticles

The aminoglycoside streptomycin was first developed in 1944, followed by others with similar chemical structure. In contrast to β-lactam antibiotics, aminoglycosides are highly effective against Gram-negative bacteria.(Mingeot-Leclercq, Glupczynski et al. 1999) They are classified as protein synthesis inhibitors which kill bacteria by binding irreversibly to the 30S subunit of the ribosomal decoding site. This action leads to an inaccurate mRNA translation and immature protein formation. Immature cell membrane proteins cause changes in cell permeability.(Hermann 2007) There are two known mechanisms by which bacteria gain resistance to aminoglycoside antibiotics. Genetic alterations lead to changes in membrane proteins which result in a lower permeability to surrounding metabolites. The second mechanism of resistance proceeds via production of aminoglycoside-modifying enzymes which covalently modify amino- and hydroxyl-functions on the aminoglycoside antibiotic. This reduces the binding affinity of the drug and prevents it from inhibiting the bacteria's ribosome. *Enterobacteriaceae* bacteria are commonly known to possess the gene causing this modification.(Mingeot-Leclercq, Glupczynski et al. 1999)

A number of studies have reported increased activities for aminoglycoside antibiotics complexed with silver nanoparticles against over fifteen strains, with synergistic increases reported for at least five of these (Table 3). Streptomycin, kanamycin and gentamicin complexes performed with comparable activity against *B. subtilis, E. coli, S. aureus* and *K.*

pneumoniae with enhancements ranging from 5% to 25%.(Baker, Pasha et al. 2015) Other studies with divergent synthesis methods for the nanoparticles reporting increases in activity of up to 57% for gentamicin against *S. aureus*.(Dhas, Mukherjee et al. 2013, Manikprabhu and Lingappa 2013) When directly compared, streptomycin exhibited the greatest improvement in activity against *E. coli* strains, kanamycin against *P, aeruginosa* and amikacin with a moderate increase compared to the two others but with a broad spectrum of activity.(Ghosh, Patil et al. 2011) For other studies FICI values were calculated for silver complexes of kanamycin(Hwang, Hwang et al. 2012) and gentamicin,(Smekalova, Aragon et al. 2016, Wang, Tang et al. 2016) with both antibiotics being similarly active against *S. aureus* and *E. coli*. Kanamycin combined with silver nanoparticles exhibited synergistic effects against *E. coli*, *K. pneumoniae*, *S. aureus* and *B. subtilis* (FICI of 0.12).(Wypij, Czarnecka et al. 2018) Gentamicin combined with nanocomposites of silver and titanium-nanotubes produced synergistic effects against *S. aureus* (FICI of 0.33) and MRSA (FICI of 0.15)(Xu, Cheng et al. 2017), or with silver nanoparticles synthesized by reduction of $AgNO_3$ in the presence of histidine and against *K. pneumoniae* (FICI of 0.15).(Kumar, Bansal et al. 2018) Within this study, histidine was found the most potent amino acid for combinations of amino acid-capped silver nanoparticles and different antibiotics with MIC values up to 0.9 mg/mL against *K. pneumoniae* and *P, aeruginosa*, 1.8 mg/mL against *E. coli and S. aureus*, and action against drug resistant clinical strains (*K. pneumoniae, S. epidermidis, S. aureus*).(Kumar, Bansal et al. 2018)

6.3. Chloramphenicol, macrolide and tetracycline antibiotics and silver nanoparticles

Chloramphenicol antibiotics were commercialized in the year 1949, originally isolated from *S. venezuelae* strains.(Smadel, Woodward et al. 1949) Similar to aminoglycosides, these antibiotics function by inhibiting protein synthesis, but they bind to the 50S ribosomal subunit, not the 30S. The mechanism of chloramphenicol is similar to macrolides in binding to the 50S ribosome.(Joseph, Al-Hakami et al. 2015) However, chloramphenicol prevents the proper binding of ribosomal substrates whereas the macrolides prevent elongation of the peptide strand.(Wolfe and Hahn 1965)

One resistance mechanism that bacteria have developed against chloramphenicol and macrolide antibiotics is to alter the 50S ribosomal subunit.(Tenson, Lovmar et al. 2003) Bacteria can also adapt by reducing membrane permeability. For chloramphenicol, bacteria can also produce chloramphenicol acetyltransferase which covalently inactivates chloramphenicol molecules.(Miyamura, Ochiai et al. 1977)

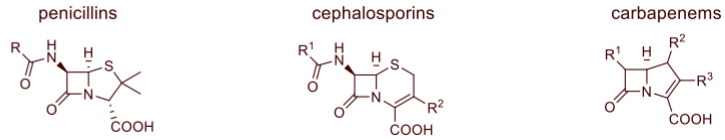
The combination of chloramphenicol and silver nanoparticles showed synergistic effects against *E. faecium*, *S. mutans, E. coli* and *P, aeruginosa.*(Hwang, Hwang et al. 2012) Furthermore, an increased activity was observed against *S. aureus, K. pneumoniae* and other bacteria strains.(Hari, Thomas et al. 2014) The increases were rather small (around 20%) compared to the values reported for β-lactam or aminoglycoside antibiotics. The highest increase was observed against *P, aeruginosa*.(Ghosh, Patil et al. 2011) Erythromycin showed

an improved action against eight bacteria strains with a maximum of 100% inhibition against *A. baumannii*.(Ghosh, Patil et al. 2011)

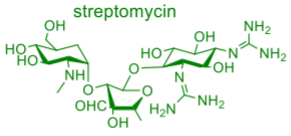
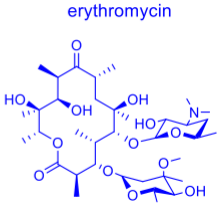
The activity of tetracycline against *E. coli, K. pneumoniae, S. aureus* and *B. subtilis* was enhanced with silver nanoparticles synthesized by *S. xinghaiensis*.(Wypij, Czarnecka et al. 2018) Furthermore, the activity of both vancomycin and rifampicin was improved against methicillin resistant *S. aureus* (FICI of 0.38 and 0.25, respectively) when combined with silver-loaded nanotubes.(Xu, Cheng et al. 2017) Thioglycolic acid-stabilized silver nanoparticles functionalized with vancomycin on the terminal carboxyl of thioglycolic acid exerted antimicrobial activity against methicillin resistant *S. aureus* (MIC of 0.05 μg/mL), vancomycin resistant *E. faecalis* (MIC of 0.1 μg/mL) and *S. epidermidis* (MIC below 0.02 μg/mL).(Esmaeillou, Zarrini et al. 2017)

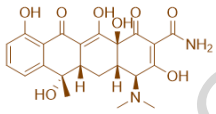
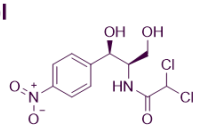
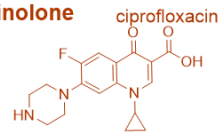
Other antibiotic classes that have been combined with silver nanoparticles include the fluoroquinolone ciprofloxacin, which showed synergistic effects against multi-resistant β-lactamase and carbapenemase producing *Enterobacteriaceae*.(Panáček, Smékalová et al. 2016) Combining other antibacterial quinolones (compounds based on a 4-oxo-1,4-dihydroquinolone skeleton) with silver complexes also revealed increased antibacterial and antifungal properties.(Rusu, Hancu et al. 2017)

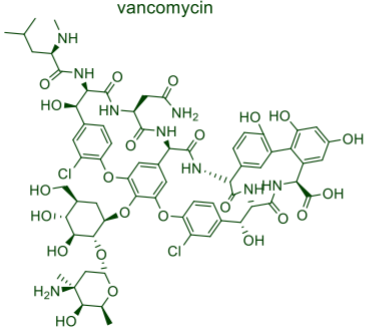
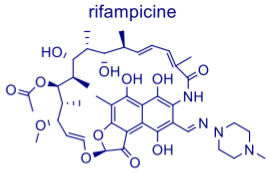
Table 3: Overview of complexes between antibiotics and silver nanoparticles, synergistic effects against specific bacteria strains, silver nanoparticle (AgNP) synthesis and the size/morphology.

Antibiotic	AgNP synthesis method	AgNP size [nm]/ morphology / concentration [μg mL^{-1}]	Bac. Strains tested – activity observed	Ref.
β–lactam antibiotics 				
Penicillin	*Plant-Assisted*: Ag^+ reduced with *D. bulbifera* tuber extract.	8-20 / mostly spherical, nanorods, triangular / 30 μg per disc	*A. baumannii* – 86; *E. cloacae* – 71; *K. pneumoniae* – 128; *N. mucosa* – 4; *P. aeruginosa* – 86; *S. typhi* – 33; *B. subtilis* – 57; *P. koreensis* – 57 *% increase	(Ghosh, Patil et al. 2011)
	Tollens process: Reduction of $[Ag(NH_3)_2]^+$ by D-maltose	26 / spherical / 0.6	*S. aureus* – synergistic effects (0.6 mg/L) *MIC	(Panáček, Smékalová et al. 2015)
	Chemical: $[Ag(NH_3)_2]^+$ by D-maltose	8 / spherical / 6.3	*A. pleuropneumoniae* – 0.3 *FICI	(Smekalova, Aragon et al. 2016)
	Reduction of $AgNO_3$ on unannealed TiO_2 nanotubes by irradiation with UV light	20-40 / spherical / 0.125-0.5	*E. coli* – 0.5 (partial synergistic); *S. aureus* – 0.516 (partial synergistic); MRSA – 0.5 *FICI	(Xu, Cheng et al. 2017)
Ampicillin	Dissolving and precipitation of solid silver.	3 / spherical / 2-8	*E. faecium* – 0.38; *S. aureus* – 0.5; *S. mutans* – 0.38; *E. coli* – 0.5; *P. aeruginosa* – 0.5 *FICI	(Hwang, Hwang et al. 2012)
	Fungi-Assisted: *T. viride* provided with $AgNO_3$ precursor.	5-30 / spherical / 10μg per disk	*E. coli* – 75; *S. typhi* – 82; *S. aureus* – 73; *M. luteus* – 70 *% increase	(Fayaz, Balaji et al. 2010)
	Plant-Assisted: Ag^+ reduced with *D. bulbifera* tuber extract.	8-20 / spherical, nanorods, triangular / 30 μg per disc	*A. baumannii* – 71; *K. pneumoniae* – 114; *N. mucosa* – 127; *P. aeruginosa* – 100; *S. typhi* – 35; *V. parahaemolyticus* – 8; *B. subtilis* – 70; *P. koreensis* – 18; *S. aureus* – 6 *% increase	(Ghosh, Patil et al. 2011)
	Tollens process: Reduction of $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 2.5	*E. coli* – synergistic effect showed by MIC of 0.03 mg/L *presented MIC values	(Panáček, Smékalová et al. 2015)
	Bacteria-assisted: *S. xinghaiensis* with $AgNO_3$ precursor.	5-20 / spherical and polydispersed / 0.004-0.12	*E. coli; K. pneumoniae*, *S. aureus*, *B. subtilis* – 0.12 *FICI	(Wypij, Czarnecka et al. 2018)
Amoxicillin	*Chemical*: $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 12.5	*A. pleuropneumoniae* – 0.4 *FICI	(Smekalova, Aragon et al. 2016)
	Plant-Assisted: Ag^+ reduced with *D. bulbifera* tuber extract.	8-20 / spherical, nanorods, triangular / 30 μg per disc	*A. baumannii* – 29; *E. cloacae* – 14; *K. pneumoniae* – 20; *P. aeruginosa* – 71; *S. typhi* – 71; *P. koreensis* – 43; *S. aureus* – 43 *% increase	(Ghosh, Patil et al. 2011)
Oxacillin	*Bacteria-assisted*: *S. coelicolor* provided with $AgNO_3$ precursor.	28-50 / Irregular shaped / 10	*S. aureus* – 100 *% increase	(Manikprabhu and Lingappa 2013)

	Tollens process: Reduction of $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 1.25	*S. aureus* - synergistic effects (0.03 mg/L) *MIC	(Panáček, Smékalová et al. 2015)
Piperacillin	*Plant-assisted*: Ag^+ reduced with *D. bulbifera* tuber extract.	8-20 / spherical, nanorods, triangular / 30 µg per disc	*A. baumannii* – 114; *S. typhi* – 20; *B. subtilis* – 40; *P. koreensis* – 114 *% increase	(Ghosh, Patil et al. 2011)
	Tollens process: Reduction of $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 2.5	*P. aeruginosa* – synergistic effects (0.015 mg/L) *MIC	(Panáček, Smékalová et al. 2015)
Cefazolin	1) *Plant-assisted*: $AgNO_3$ reduced with *A. sativum* extract. 2) *Chemical*: $AgNO_3$ reduction with sodium citrate.	1) 14 /spherical / 20; 2) 12 / spherical / 20	*L. lactis* – 14; *M. luteus* – 30; *E. coli* – 3.4; *P. aeruginosa* – 15; *L. lactis* – 7; *M. luteus* – 10; *P. vulgaris* – 3.2 *% increase	(Hari, Thomas et al. 2014)
	Tollens process: Reduction of $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 2.5	*E. coli* – synergistic effects (0.0019 mg/L) *MIC	(Panáček, Smékalová et al. 2015)
Ceftriaxone	*Plant-assisted*: Ag^+ reduced with *D. bulbifera tuber* extract.	8-20 / spherical, nanorods, triangular/ 30 µg per disc	*H. influenza* – 15; *S. typhi* – 20; *V. parahaemolyticus* – 20 *% increase	(Ghosh, Patil et al. 2011)
Cefotaxime	*Plant-assisted*: $AgNO_3$ reduced with *T. angustifolia* leave extract.	8 / spherical / 1.4	*E. coli* – 63; *K. pneumonia* – 63 *% increase	(Gurunathan 2015)
	Plant-assisted: Ag^+ reduced with D. bulbifera tuber extract.	8-20 / spherical, nanorods, triangular / 30 µg per disc	*A. baumannii* – 30; *H. influenza* – 21; *S. typhi* – 11; *V. parahaemolyticus* – 13; *B. subtilis* – 7 *% increase	(Ghosh, Patil et al. 2011)
	Tollens-process: Reduction of $AgNO_3$ with NaOH and D-maltose, stabilized by gelatin (0.05%)	28 nm / spherical / 0.4-3.4	ESBL-positive *E. coli* – 0.4; AmpC-positive *E. coli* – 0.28; ESBL-positive *K. pneumoniae* – 0.18 *FICI	(Panáček, Smékalová et al. 2016)
Cefuroxime	*Tollens process*: Reduction of $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 2.5	*E. coli* – synergistic effects (0.00097 mg/L) *MIC	(Panáček, Smékalová et al. 2015)
Cefoxitin	*Tollens process*: Reduction of $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 2.5	*E. coli* – synergistic effects (0.00097 mg/L) *MIC	(Panáček, Smékalová et al. 2015)
Cefepime	*Tollens process*: Reduction of $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 2.5	*P. aeruginosa* – synergistic effects (0.00024 mg/L) *MIC	(Panáček, Smékalová et al. 2015)
Cefoperazone	*Tollens process*: Reduction of $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 2.5	*P. aeruginosa* – synergistic effects (0.00024 mg/L) *MIC	(Panáček, Smékalová et al. 2015)
Ceftazidime	*Plant-assisted*: Ag^+ reduced with D. bulbifera tuber extract.	8-20 / spherical, nanorods, triangular / 30 µg per disc	*E. coli* – 100; *S. odorifera* – 4; *P. koreensis* – 6; *S. aureus* – 25 *% increase	(Ghosh, Patil et al. 2011)
	Chemical: $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 2.5	*P. aeruginosa* – synergistic effects (0.00024 mg/L) *MIC	(Panáček, Smékalová et al. 2015)
	Tollens-process: Reduction of $AgNO_3$ with NaOH and D-maltose, stabilized by gelatin (0.05%)	28 nm / spherical / 0.4-3.4	ESBL-positive *E. coli* – 0.36; AmpC-positive *E. coli* – 0.28; KPC-positive *K. pneumoniae* – 0.38; ESBL-positive *K. pneumoniae* – 0.2 *FICI	(Panáček, Smékalová et al. 2016)

Cephalothin	1) *Plant-assisted*: $AgNO_3$ reduced with *A. sativum* extract. 2) *Chemical*: $AgNO_3$ reduction with sodium citrate.	1) 14 /spherical / 20; 2) 12 / spherical / 20	1) *S. aureus* – 9; *M. luteus* – 18; *B. subtilis* – 30; *P. mirabilis* – 5.8. 2) *P. aeruginosa* - 7.1; *L. lactis* - 7.1; *B. subtilis* - 30; *S. typhi* - 7.6; *P. mirabilis* -2.9 *% increase	(Hari, Thomas et al. 2014)
Meropenem	*Plant-assisted*: Ag^+ reduced with *D. bulbifera* tuber extract.	8-20 / spherical, nanorods, triangular / 30 µg per disc	*K. pneumoniae* – 114; *P. koreensis* – 5 *% increase	(Ghosh, Patil et al. 2011)
	Tollens-process: Reduction of $AgNO_3$ with NaOH and D-maltose, stabilized by gelatin (0.05%)	28 nm / spherical / 0.4-3.4	KPC-positive *K. pneumoniae* – 0.37 *FICI	(Panáček, Smékalová et al. 2016)
aminoglycosides  **macrolides** 				
Strepto-mycin	*Bacteria-assisted*: *P. veronii* provided with $AgNO_3$ and $HAuCl_4$ precursor.	30 /mixture / 1	*B. subtilis* – 3.8; *E. coli* – 20; *S. aureus* – 9.5; *K. pneumoniae* – 7.1 *% increase	(Baker, Pasha et al. 2015)
	Plant-assisted: Ag^+ reduced with *D. bulbifera* tuber extract.	8-20 / spherical, nanorods, triangular / 30 µg per disc	*E. coli* – 257; *P. aeruginosa* – 33; *S. typhi* – 43 *% increase	(Ghosh, Patil et al. 2011)
Neomycin	*Chemical*: $AgNO_3$ reduced with sodium citrate.	23 / / 5	*S. typhimurium* – not quantified, but noted synergistic increase.	(McShan, Zhang et al. 2015)
Kanamycin	*Plant-assisted*: Ag^+ reduced with *D. bulbifera* tuber extract.	8-20 / spherical, nanorods, triangular / 30 µg per disc	*A. baumannii* – 42; *E. coli* – 14; *K. pneumoniae* – 29; *P. aeruginosa* – 129; *S. typhi* – 43; *V. parahaemolyticus* – 5; *P. koreensis* – 10; *S. aureus* – 6 *% increase	(Ghosh, Patil et al. 2011)
	Fungi-Assisted: *T. viride* provided with $AgNO_3$ precursor.	5-30 / spherical / 10 µg per disk	*E. coli* – 33; *S. typhi* – 46; *S. aureus* – 22; *M. luteus* – 10 *% increase	(Fayaz, Balaji et al. 2010)
	Dissolving and precipitation of solid silver.	3 / spherical / 2-4	*E. faecium* – 0.75; *S. aureus* – 0.38; *S. mutans* – 0.38; *E. coli* – 0.5; *P. aeruginosa* – 0.38 *FICI	(Hwang, Hwang et al. 2012)
	Bacteria-assisted: P. *veronii* provided with $AgNO_3$ and $HAuCl_4$ precursor.	30 / mixture / 1	*B. subtilis* – 12; *E. coli* – 12; *S. aureus* – 8.6; *K. pneumoniae* – 25 *% increase	(Baker, Pasha et al. 2015)
	Bacteria-assisted: *S. xinghaiensis* with $AgNO_3$ precursor.	5-20 / spherical and polydispersed / 0.125-32	*E. coli; K. pneumoniae*, *S. aureus*, *B. subtilis* – 0.12 *FICI	(Wypij, Czarnecka et al. 2018)
Amikacin	*Plant-assisted*: Ag^+ reduced with *D. bulbifera* tuber extract.	8-20 / spherical, nanorods, triangular / 30 µg per disc	*E. cloacae* – 5; *E. coli* – 80; *N. mucosa* – 30; *P. aeruginosa* – 57; *S. typhi* – 14; *P. koreensis* – 33; *S. aureus* – 27 *% increase	(Ghosh, Patil et al. 2011)
	Tollens process: Reduction of $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 2.5	*P. aeruginosa* – synergistic effects (0.00048 mg/L) *MIC	(Panáček, Smékalová et al. 2015)

Gentamicin	*Bacteria-assisted*: *P. veronii* provided with $AgNO_3$ and $HAuCl_4$ precursor.	30 / mixture /1	*B. subtilis* – 3.1; *E. coli* – 15; *S. aureus* – 4; *K. pneumonae* – 22 *% increase	(Baker, Pasha et al. 2015)
	Bacteria-assisted: *S. coelicolor* provided with $AgNO_3$ precursor.	28-50 / Irregular shaped / 1	*S. aureus* – 57 *% increase	(Manikprabhu and Lingappa 2013)
	Plant-assisted: $AgNO_3$ reduced with *R. apiculata* extract.	6-15 / Spherical / 30 µg per disk	*B. cereus* – 25; *S. aureus* – 44; *P. mirabilis* – 11; *E. coli* – 14 *% increase	(Dhas, Mukherjee et al. 2013)
	PVP coated silver NP	37-39 / spherical	*E. coli* – 0.37; *S. aureus* – 0.28 *FICI	(Wang, Tang et al. 2016)
	Chemical: $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 3 - 25	*E. coli* – 0.4; *S. aureus* – 0.4; *A. pleuropneumoniae* – 0.5 *FICI	(Smekalova, Aragon et al. 2016)
	Tollens process: Reduction of $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 1.25	*E. coli* – synergistic effects (0.06 mg/L); *S. aureus* – synergistic effects (0.015 mg/L) *MIC	(Panáček, Smékalová et al. 2015)
	Reduction of $AgNO_3$ on unannealed TiO_2 nanotubes by irradiation with UV light	20-40 / spherical / 0.125-0.25	*S. aureus* – 0.325 (partial synergistic); MRSA – 0.15 *FICI	(Xu, Cheng et al. 2017)
	Tollens-process: Reduction of $AgNO_3$ with NaOH and D-maltose, stabilized by gelatin (0.05%)	28 nm / spherical / 0.4-3.4	ESBL-positive *E. coli* – 0.5; AmpC-positive *E. coli* – 0.48; ESBL-positive *K. pneumoniae* – 0.33 *FICI	(Panáček, Smékalová et al. 2016)
	Reduction of $AgNO_3$ by aqueous solution of histidine	8-45 / spherical / 0.05	*K. pneumoniae* – 0.152 *FICI	(Kumar, Bansal et al. 2018)
Erythro-mycin	*Bacteria-assisted*: *P. veronii* provided with $AgNo_3$ and $HAuCl_4$ precursor.	~30 / mixture / 1	*B. subtilis* – 8.8; *E. coli* – 8; *S. aureus* - 6.3; *K. pneumoniae* - 16 *% increase	(Baker, Pasha et al. 2015)
	Plant-assisted: Ag^+ reduced with *D. bulbifera* tuber extract.	~8-20 / anisotropic but mostly spherical, nanorods and triangular / 30 µg per disc	*A. baumannii* – 100; *E. cloacae* – 14; *E. coli* – 14; *K. pneumoniae* – 57; *N. mucosa* – 8; *P. mirabilis* – 57 *% increase	(Ghosh, Patil et al. 2011)
tetracycline  **chloramphenicol**  **fluorochinolone** ciprofloxacin 				
Tetracycline	*Bacteria-assisted*: *S. xinghaiensis* with $AgNO_3$ precursor.	5-20 / spherical and polydispersed / 0.008-16	*E. coli; K. pneumoniae*, *S. aureus*, *B. subtilis* – 0.12 *FICI	(Wypij, Czarnecka et al. 2018)
	Tollens process: Reduction of $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 0.6-5	*S. aureus* – synergistic effects (0.015 mg/L); *E. coli* – synergistic effects (0.00048 mg/L) *MIC	(Panáček, Smékalová et al. 2015)
Chloramph-enicol	*Bacteria-assisted*: *P. veronii* provided with $AgNo_3$ and $HAuCl_4$ precursor.	~30 / mixture / 1	*B. subtilis* - 3.3; *E. coli* - 6.6; *S. aureus* – 16; *K. pneumoniae* – 12 *% increase	(Baker, Pasha et al. 2015)
	Dissolving and precipitation of solid silver.	~3 / spherical / 1-4	*E. faecium* – 0.34; *S. aureus* – 0.75; *S. mutans* – 0.38; *E.coli* – 0.5; *P. aeruginosa* - 0.38 *FICI	(Hwang, Hwang et al. 2012)

	1) *Plant-assisted*: $AgNO_3$ reduced with *A. sativum* extract. 2) *Chemical*: $AgNO_3$ reduction with sodium citrate.	1) 14 / spherical / 20,2) 12 / spherical / 20	*E.coli* – 28; *P. aeruginosa* – 16; *S. aureus* – 3.5; *L. lactis* – 20; *M. luteus* – 11; *B. subtilis* – 25; *P. vulgaris* – 3.3 * % increase	(Hari, Thomas et al. 2014)
	Fungi-assisted: *T. viride* provided with $AgNO_3$ precursor.	~5-30 / spherical / 10 µg per disk	*E.coli* – 27; *S. typhi* – 24; *S. aureus* – 11; *M. luteus* - 10 *% increase	(Fayaz, Balaji et al. 2010)
	Plant-assisted: Ag^+ reduced with *D. bulbifera* tuber extract.	~8-20 / anisotropic but mostly spherical, nanorods and triangular / 30 µg per disc	*K. pneumoniae* – 71; *P. aeruginosa* – 142; *S. thypi* – 9; *B. subtilis* – 33; *S. aureus* – 43 *% increase	(Ghosh, Patil et al. 2011)
	Plant-assisted: $AgNO_3$ reduced with *R. apiculata* leave extract.	~15 / spherical.	*B. cereus* – 23; *S. aureus* – 19; *P. mirabilis* - 7.7; *E. coli* - 11 *% increase	(Dhas, Mukherjee et al. 2013)
	Tollens process: Reduction of $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 2.5	*S. aureus* – synergistic effects (0.00024 mg/L) *MIC	(Panáček, Smékalová et al. 2015)
Ciprofloxacin	Tollens-process: Reduction of $AgNO_3$ with NaOH and D-maltose, stabilized by gelatin (0.05%)	28 nm / spherical / 0.4-3.4	ESBL-positive *E. coli* – 0.22; AmpC-positive *E. coli* – 0.34; KPC-positive *K. pneumoniae* – 0.38; ESBL-positive *K. pneumoniae* – 0.33 *FICI	(Panáček, Smékalová et al. 2016)
glycopeptide vancomycin  **ansamycine** rifampicine 				
Vancomycin	Reduction of $AgNO_3$ on unannealed TiO_2 nanotubes by irradiation with UV light	20-40 / spherical / 0.125-0.25	*S. aureus* – 0.5 (partial synergistic); MRSA – 0.375 *FICI	(Xu, Cheng et al. 2017)
	Tollens process: Reduction of $[Ag(NH_3)_2]^+$ by D-maltose	28 / spherical / 1.25	*S. aureus* – 0.25 *FICI calculated with given MICs	(Panáček, Smékalová et al. 2015)
Rifampicin	Reduction of $AgNO_3$ on unannealed TiO_2 nanotubes by irradiation with UV light	20-40 / spherical / 0.125	MRSA – 0.25 *FICI	(Xu, Cheng et al. 2017)

7. Synergistic effects between silver nanoparticles and other compounds

There are also silver complexes exerting synergistic effects on bacteria. Silver-*N*-heterocyclic carbene complexes showed an inhibition of bacterial growth (*S. aureus, S. enterica*).(Aher, Das et al. 2017) Silver nanoparticles synthesized and stabilized by metabolites of *T. viride* were potent against *S. aureus* due to an increased production of reactive oxygen species.(Kumari, Shukla et al. 2017) Binary silver(I) complexes with either nicotinamide or glycine showed a growth inhibition effect (higher in case of silver nicotinamide) and an increased antimicrobial activity against *S. aureus* and *E. coli*.(Rendošová, Vargova et al. 2017) Using titanium dioxide and silver composites, a high bacteriostatic rate against *E. coli*(Wang, Chen et al. 2017, Ye, Cheng et al. 2017) and *S. aureus*(Ye, Cheng et al. 2017) was achieved again exploiting synergistic effects of reactive oxygen species and released silver ions.(Ye, Cheng et al. 2017) Combination of polycationic polymers and silver cations revealed an antimicrobial material with activity against *E. coli* and *S. aureus*.(Guo, Xu et al. 2017)

The combination of two or more components with synergistic effects is not necessarily always the best solution, as microbes can still develop resistance over a long exposure period.(Graves, Thomas et al. 2017) An alternative approach is to combine various components demonstrating antagonistic effects, especially with one of the antimicrobial agents being an essential metal, for example combinations of iron and silver. Bacterial response is developing their resistance to toxic silver by eliminating it, which consequently leads to reducing the iron level in the cell. If the element is required by bacteria, but is eliminated by a resistance mechanism, the bacteria will facilitate their own death.(Graves, Thomas et al. 2017) Such reflection highlights the importance of understanding the biology behind the inhibition mechanisms and microbes' behavior, their enormous potential to quickly adapt in new environment by activating silent genes from their rich repertoire.

8. Remarks, perspective and prognosis

There are now an increasing number of reports regarding the use of silver against multi-drug resistance bacteria that cover investigations into its mode of action, proposed mechanisms of antibiotic-resistance and the potential synergistic effects of complexes with antibiotics. Silver, when used in conjunction with antibiotics, often improves antimicrobial activity and can 'resuscitate' older antibiotics that are no longer efficacious due to the development of resistance.

However, as bacterial resistance against silver nanoparticles has recently been described(Panáček, Kvítek et al. 2018), there is a danger that this may spread as a result of the increased quantity of commercially available products incorporating silver nanoparticles.(Gunawan, Marquis et al. 2017) Indeed, nanoparticles have been already detected in animal products consumed by humans.(Gallocchio, Biancotto et al. 2017) This resistance may be avoided by formulating the nanoparticles with antimicrobial agents that have synergistic effects, to reduce the chance of a stochastic response by microbes evolving a single mechanism of resistance.(Graves, Thomas et al. 2017, Reshma, Syama et al. 2017)

Using combinations of silver and antibiotics could re-invigorate current drugs that have lost their effectiveness due to the development of resistant microbes. If such combinations are approved, it will be prudent to control and monitor usage of silver nanoparticles, both in the prescription of therapeutics in a clinical setting and everyday health supplements and consumer products.

Silver and silver ions cannot be treated as a 'cure-all' or 'stand-alone' therapies, but silver coupled with antimicrobial agents warrant re-examination as potential investigational drug candidates. While silver has been shown to be cytotoxic to mammalian cells in some cases, recent research in orthopedic implants suggests silver nanoparticles as promising candidates for rational therapy design and prevention option for bacterial bone and infections.(Aurore, Caldana et al. 2018) Silver has been used in alternative health supplements with no significant adverse clinical findings at low doses(Mathur, Jha et al. 2017).

The wider commercial availability of antimicrobial silver products necessitates better controls, vigilance and monitoring of clinical and environmental contamination of a potent antimicrobial agent. Overuse and misuse of antibiotics expedites the development of resistant superbugs. Future alternative treatments may include targeted therapies that incorporate synergistic incorporation of silver. With a paucity of new antibiotics in the development pipeline and even fewer truly novel chemotypes being discovered in the last decade, we should also explore combining 'ineffective' antibiotics with adjuvants, such as silver, to create antibiotic-metal combinations that address the dire global threat of drug-resistant infections.

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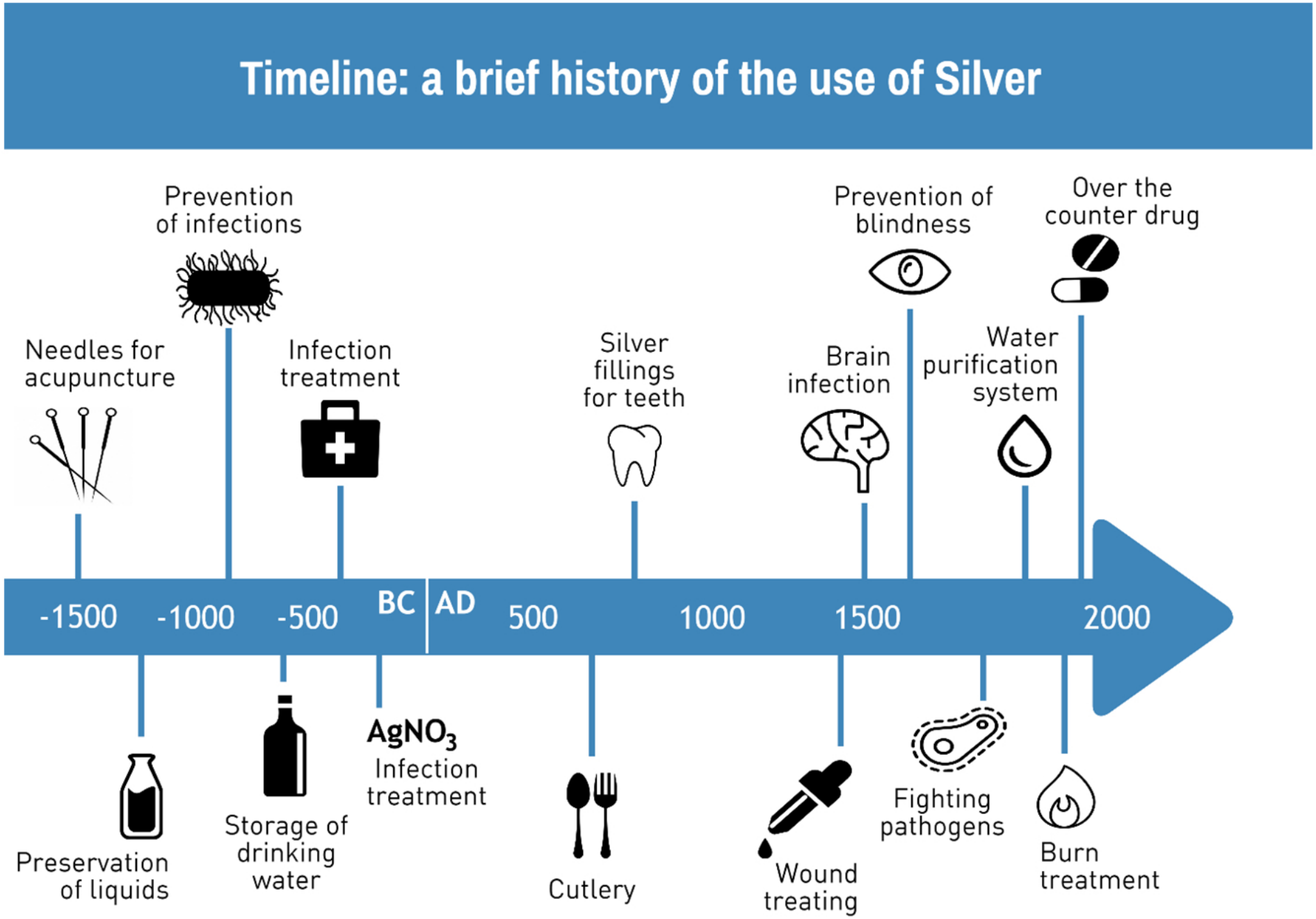
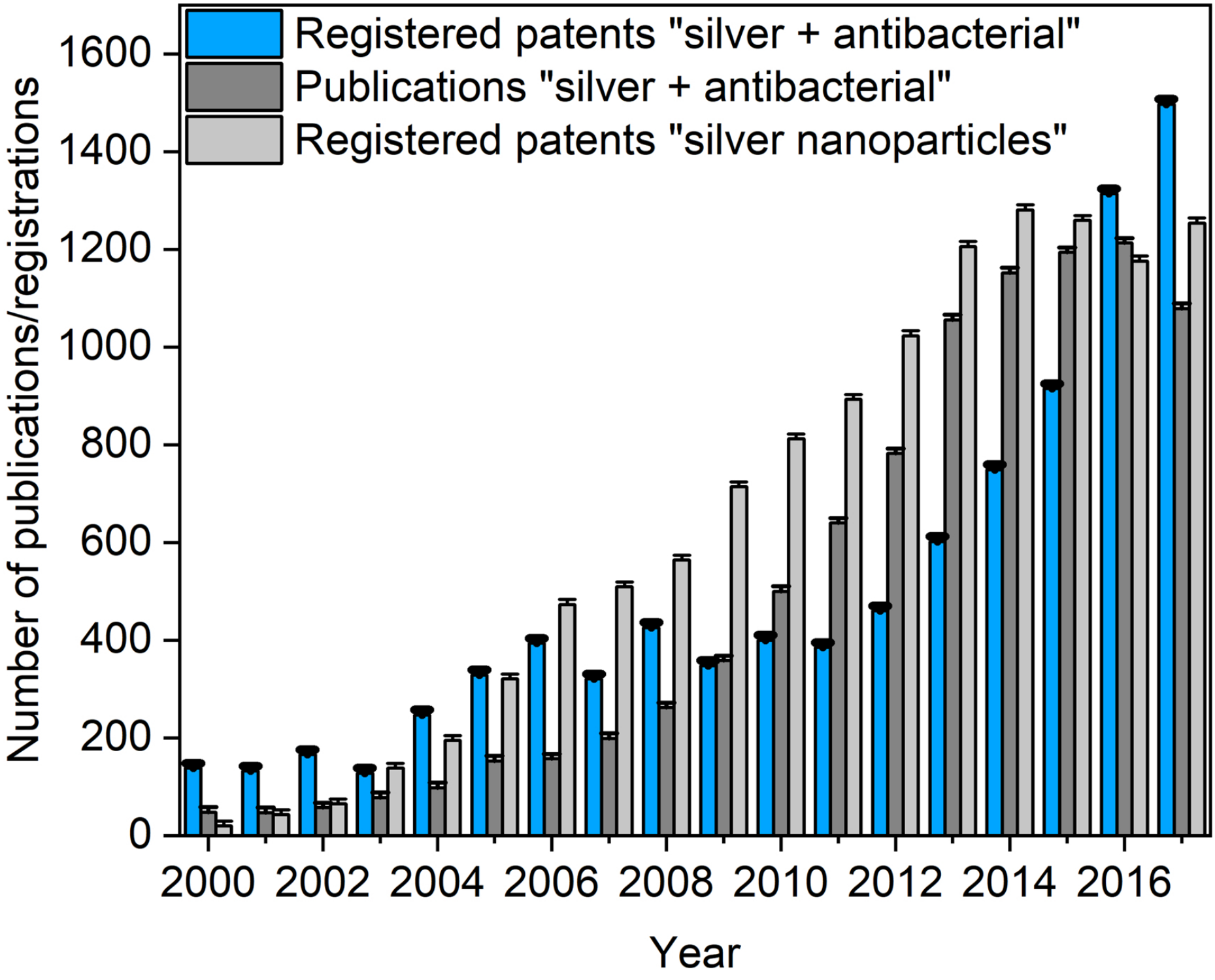

Timeline: a brief history of the use of Silver
Prevention of infections
Needles for acupuncture
Infection treatment
Silver fillings for teeth
Prevention of blindness
Brain infection
Water purification system
Over the counter drug
-1500
-1000
-500
BC
AD
500
1000
1500
2000
Preservation of liquids
Storage of drinking water
AgNO3 Infection treatment
Cutlery
Wound treating
Fighting pathogens
Burn treatment

Figure 1


Registered patents "silver + antibacterial"
Publications "silver + antibacterial"
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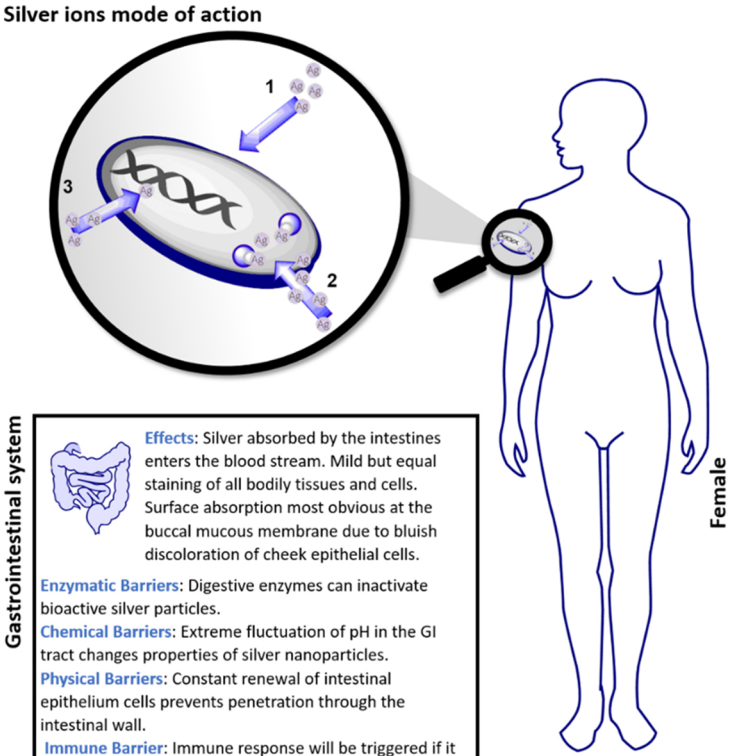
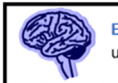
Year

Figure 2



Brain

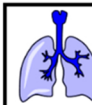


Effects: Deposition in brain is still unknown.

Physical Barrier: Highly selective membrane which only allows exchange of gasses and lipid soluble particles.

Immune Barriers: Certain forms of silver will trigger the onset of immune response.

Respiratory System



Effects: Known to cause oxidative stress to lung epithelial cells with generation of radicals and ROS, cytotoxic to alveolar macrophage cells.

Physical Barrier: Particles >2.5 μm trapped in the mucus layer and expelled by ciliary actions in the nasal passage.

Lymphatic Drainage: Accumulation of on-site immune effectors and circulation to liver to be detoxified

Female urogenital

Immune Barriers: Mucosal lining with immune infector cells, on-site secretion of IgG

Skin

Effect: Nanoparticles can be internalized by epidermal keratinocytes, setting up an immune response, toxic to fibroblast and keratinocytes. Argyria occurring locally on site of exposure.

Physical Barriers: Outermost layer is strengthened by keratin. Selective permeability only allowing lipophilic particles to pass through.

Enzymatic Barriers: Proteolytic break down of any silver-protein complexes.

Gastrointestinal system

Effects: Silver absorbed by the intestines enters the blood stream. Mild but equal staining of all bodily tissues and cells. Surface absorption most obvious at the buccal mucous membrane due to bluish discoloration of cheek epithelial cells.

Enzymatic Barriers: Digestive enzymes can inactivate bioactive silver particles.

Chemical Barriers: Extreme fluctuation of pH in the GI tract changes properties of silver nanoparticles.

Physical Barriers: Constant renewal of intestinal epithelium cells prevents penetration through the intestinal wall.

Immune Barrier: Immune response will be triggered if it is absorbed into the lymphatic system.

Figure 3