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Cellular and Molecular Impact of Green Synthesized Silver Nanoparticles

Paritosh Patel, Puja Kumari, Suresh K. Verma and M. Anwar Mallick

Abstract

Toxicity and biocompatibility of silver nanoparticles are of a major concern due to their extensive production regardless of their application in current industries. Information about toxicology or biocompatibility is crucial regarding their proper utilization and application in clinical as well as environmental aspect. This chapter describes in detail about the different techniques and technology of synthesis of silver nanoparticles and explains their different physiochemical properties in context of the current research scenario. Further, it also explains the biocompatibility and toxicity of silver nanoparticles at cellular and molecular aspects. The mechanism of their toxicity has been described keeping in view of the recent research done. In brief, it reveals detail knowledge of the cellular and molecular impact of silver nanoparticles.

Keywords: silver nanoparticles, toxicology, oxidative stress, apoptosis

1. Introduction

Really revolutionary nanotech items, materials and application for example nanorobotics, are years long in the future. But what qualifies as “Nanotechnology” today is fundamental innovation that is going on in research centers everywhere throughout the world. Products of Nanotech which are on business sector today are generally steadily improved products (utilizing evolutionary nanotechnology) where some types of Nano-empowered materials (for example, Carbon nanotubes, nanocomposite structure of nanoparticles of specific substance) or nanotech process (for example Nano-patterning or Quantum Dots for medicinal imaging) is utilized in the assembling procedure. In their progressing and ongoing journey to improve existing products by making smaller parts and better execution materials, all at a lower cost, the number of organization that will make “Nano products” will become extremely fast and soon make up the most of all organization across numerous businesses.

Nanomaterials (NMs) have picked up noticeable quality in technological progressions due to their tunable synthetic, physical and organic properties with improved execution over their bulk counter partners. They are arranged depending on their origin, size, shape and composition. The capacity to anticipate the remarkable properties of NMs expand the estimation of each classification. Nanomaterials

speak to an active/functioning area of research and techno-economic parts in numerous application areas. NMs are depicted as a material with a length of 1–1000 nm in at least one dimension [1]. In any case, a single globally acknowledge definition for NMs does not exist. The diverse association has a distinction in assessment in defining NMs. As indicated by the Environmental Protection Agency (EPA), NMs can display remarkable properties unique than the equal chemical compound in a bigger dimension [2]. The US Food and Administration (USFDA) likewise alludes to NMs as “materials that have at least one dimension dependent phenomena” [2]. The International Organization for Standardization (ISO) has depicted NMs as a “Materials with any external nanoscale measurement or having internal nanoscale surface structure” [2]. As of late, the British Standard Institution proposed the following definition for the scientific terms that have been utilized:

- *Nanoscale*: Approximately 1–1000 nm size range [1, 3].
- *Nanoscience*: The science and investigation of matter at the nanoscale that manages to understand their size and structure-dependent properties and compares at the rise of individual atoms or molecules or bulk materials related differences [1, 3].
- *Nanotechnology*: manipulation and control of matter on a nanoscale measurement by utilizing scientific logical knowledge of different industrial and biomedical applications [1, 3].
- *Nanomaterials*: Materials with any inside or outside structure on the nanoscale measurements [1, 3].
- *Nano-objects*: Materials that have at least one or more peripheral nanoscale measurements.
- *Nanoparticles*: Nano-objects with three outer nanoscale measurements. The terms Nano rod or Nano plate are utilized, rather than nanoparticles (NP) when the longest and the shortest axes length of a nano-object are unique [1, 3].
- *Nanofiber*: When two comparable exterior nanoscale measurements and a third measurement are available in a nanomaterial, it is alluded to as a nanofiber [1, 3].
- *Nanocomposite*: Multiphase structure with at least one phase on the nanoscale measurement [1, 3].
- *Nanostructure*: Composition of interconnected parts in the nanoscale area [1, 3].
- *Nanostructured materials*: Materials containing interior or surface nanostructure [1, 3].

The nanoparticles shows remarkable chemical, physical and natural properties at nanoscale contrasted with their respective particles at higher scales. This phenomenon is because of a moderately bigger surface region to the volume, expand reactivity or stability in a synthetic procedure, improved mechanical strength and so forth. These properties of nanoparticles have prompted its utilization of different applications [3]. Nanoparticles have been utilized in medication (drug delivery), in food industries, gene delivery and Cancer therapy and so on [3]. The nanoparticles are of various size, structure and shape. It well may be tubular, conical, spherical,

hollow core, cylindrical, spiral, flat and so forth or sporadic and contrast from 1 to 100 nm in size. Nanomaterials/or nanoparticles are utilized in an expansive range of use. Today they contained in numerous products and utilized in different technologies. Most Nano items created on an industrial scale are nanoparticle, in spite of the fact that they likewise emerge as by-products in the manufacturing of other materials [4, 5]. Explicit synthesis is utilized to create the different nanoparticles, coating composite and dispersion. Characterized production and reaction condition is pivotal in acquiring such size-dependent molecule. Particle shape, crystallinity, chemical composition and size can be constrained by pH- value, synthetic arrangement (chemical), temperature, procedure control and surface modification [5].

Two fundamental procedures are utilized to create nanoparticles: “Top-down” and “Bottom-up”. The expression “Top-down” alludes here to the mechanical squashing of source materials utilizing a milling procedure. In the “Bottom-up” strategy, structures are developed by the synthetic procedure. The determination of the individual procedure relies upon the compound organization and the desire features indicated for the nanoparticles [6] (Figure 1) (Table 1).

Strangely, the morphological parameters of NPs can be tweaked by shifting the chemical concentration and reaction condition for example pH and temperature. However, if these synthesized NMs are exposed to the real application, then they can experience the following impediment, which is stability in a threatening situation, absence of comprehension in fundamental mechanism and modeling factors, bioaccumulation or toxicity quality, extensive examination requirements recycle, reuse, regeneration. In true word, it is desirable that the properties, behavior and types of nanomaterials ought to be improved to meet the aforementioned points. Then again, these impediments are opening new and extraordinary opportunities in this developing field of research.

To counter those restrictions a new era of green synthesis methodologies is increasing incredible in recent research and innovative work on material science and technologies. Essentially green synthesis will straightforwardly help uplift the ecological friendliness as they are generated through clean up, regulation/guideline, control and remediation process additionally there are few parts like the decrease of derivatives, decrease of contamination, prevention and minimalization of waste

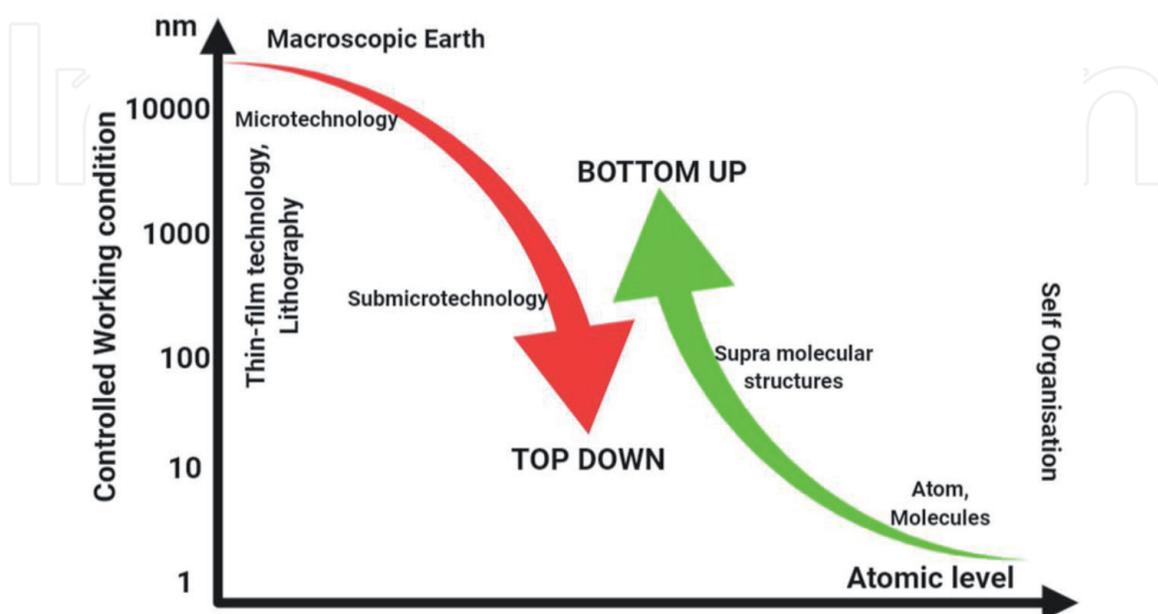


Figure 1. Methods of nanoparticles production: top-down and bottom-up (image: Laboratory for micro and nanotechnology, Paul Scherrer Institut).

Category	Method	Nanoparticles
Bottom-up	Pyrolysis	Carbon and metal oxide based
	Biosynthesis	Organic polymers and metal based
	Spinning	Organic polymers
	Sol-gel	Carbon metal and metal oxide based
	Chemical vapor deposition (CVD)	Carbon and metal based
Top-down	Sputtering	Metal based
	Laser ablation	Carbon based and metal oxide based
	Thermal decomposition	Carbon and metal oxide based
	Nanolithography	Metal based
	Mechanical milling	Metal, oxide and metal oxide based

Table 1.
Categories of the nanoparticles synthesized from the various methods [1].

and ultimately the utilization of more secure solvent during synthesis process as well as renewable stock. Green synthesis is required to stay away from the production of undesirable or unsafe products through the build-up of reliable, maintainable and eco-friendly methods. Green synthesis of metallic nanoparticles has been embraced to suit different organic material (for example, algae, bacteria, plant extract and fungi) (Figure 2) [7]. Among the accessible green methods of synthesis for metal and metal oxide NPs, usage of plant extract is a fairly straightforward and simple procedure to create nanoparticles at large scale with respect to fungi and bacteria mediated synthesis. Synthesis of metal and metal oxide NPs, plant biodiversity has been comprehensively considered to be because of the availability of effective phytochemicals in different plant extract, particular in leaves such as amide, flavones, phenols, terpenoids, ketones, ascorbic acid, aldehyde and carboxylic acids. These components are equipped of reducing metal salts into metal NPs [7, 8] (Tables 2 and 3).

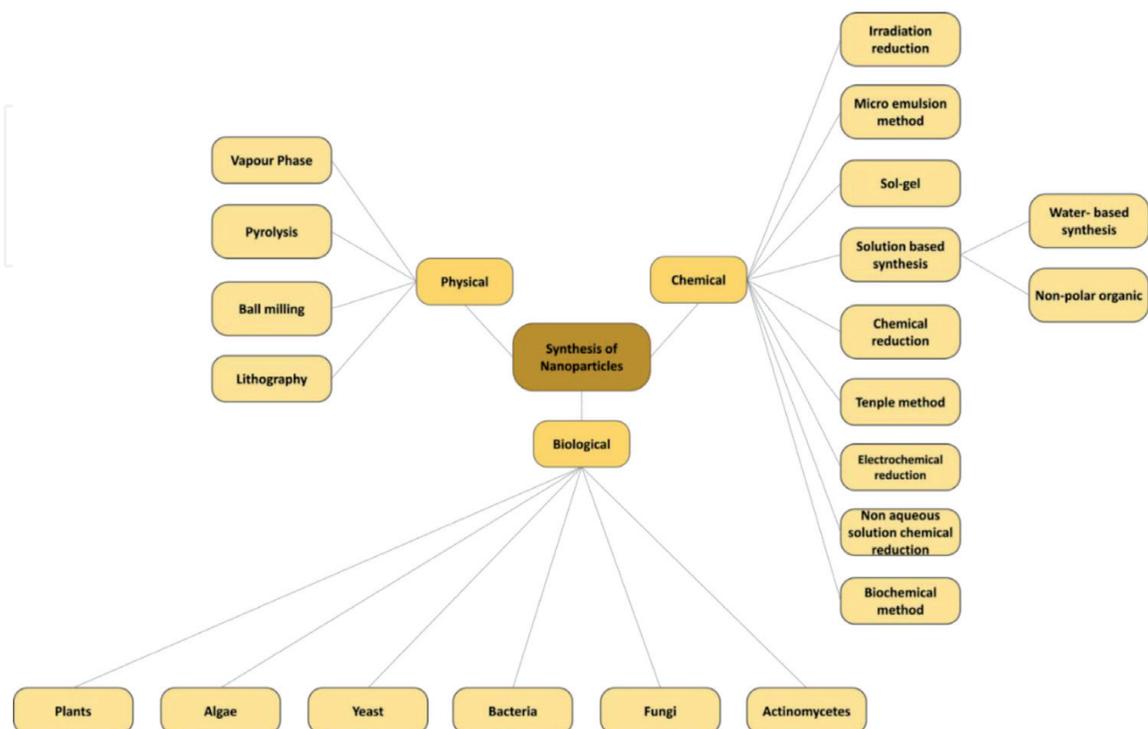


Figure 2.
Different methods for the synthesis of nanoparticles [4, 7].

Sr. no.	Species	Nanoparticles	Size (nm)	Morphology	Application
1	<i>Lactobacillus casei</i>	Silver	20–50	Spherical	Drug delivery, bio-labeling
2	<i>Desulfovibrio desulfuricans</i>	Gold	20–50	Spherical	Catalysis
3	<i>E. coli</i>	Cadmium	2–5	Fluorescent labels	Wurtzite structures
4	<i>Klebsiella pneumonia</i>	Silver	28–122	Spherical	Optical receptor, antimicrobial
5	<i>Aquaspirillum magnetotacticum</i>	Iron oxide	40–50	Octahedral prism	—
6	<i>Coriolus versicolor</i>	Silver	25–75	Spherical	Water-soluble metallic catalyst
7	<i>Penicillium brevicompactum</i>	Silver	23–105	Crystalline spherical	Antimicrobial agent
8	<i>Phoma glomerata</i>	Silver	60–80	Spherical	Antimicrobial agent
9	<i>Saccharomyces cerevisiae</i> broth	Gold, silver	4–15	Spherical	Catalysis
10	<i>Aspergillus flavus</i> TFR7	Titanium dioxide	12–15	Spherical	Plant nutrient fertilizer

Table 2.
 Synthesis of metallic nanoparticles from various biological species (bacteria) [7].

Sr. no.	Species	Nanoparticles	Size (nm)	Morphology	Application
1	<i>Eucalyptus citriodora</i> (neelagiri)	Silver	20	Spherical	Antibacterial
2	<i>Cymbopogon flexuosus</i> (lemon grass)	Gold	200–500	Spherical, triangular	Infrared-absorbing optical coating
3	<i>Syzygium aromaticum</i> (clove buds)	Gold	5–100	Irregular	Detection and destruction of cancer cells
4	<i>Mentha piperita</i> (peppermint)	Silver	5–30	Spherical	Kill microbes
5	<i>Medicago sativa</i> (alfalfa)	Gold	2–40	Hexagonal, tetrahedral, icosahedral	Labeling in structural biology, paints
6	<i>Morus</i> (mulberry)	Silver	15–20	Spherical	Antimicrobial activity
7	<i>Aloe barbadensis</i> Miller (<i>Aloe vera</i>)	Gold, silver	10–30	Spherical, triangular	Cancer hyperthermia, optical coating
8	<i>Coriandrum sativum</i> (coriander)	Gold	6.75–57.91	Spherical, triangular	Drug delivery, tumor imaging
9	<i>Azadirachta indica</i> (neem)	Gold, silver	5–35	Spherical, hexagonal	Remediation of toxic metal
10	<i>Terminalia catappa</i> (almond)	Gold	10–35	Spherical	Biomedical field

Table 3.
 Synthesis of metallic nanoparticles from various plant extract [7].

2. Classification of nanoparticles

2.1 Organic nanoparticles

Dendrimers, micelles, liposomes and ferritin are usually known as natural nanoparticles or polymers. These nanoparticles are biodegradable, non-toxic and a few particles for example, micelles and liposomes have a hollow center otherwise known as nanocapsules [9].

2.2 Inorganic nanoparticles

Inorganic nanoparticles are not comprised of carbon. Metal and metal oxide based nanoparticles are commonly classified as inorganic nanoparticles.

- *Metal based*: Nanoparticles that are integrated from metals to Nano size either by ruinous or constructive strategies are metal based nanoparticles [1, 9]. Practically every one of metal can be synthesized into their nanoparticles. The normally utilized metals for nanoparticles are aluminum, cobalt, gold, silver, zinc, iron, copper, and cobalt [1, 9]. Nanoparticles have a distinctive size extends from 10 nm to 100 nm.
- *Metal oxides based*: The metal oxides based nanoparticles are orchestrated to adjust the properties of their respective metal based nanoparticles.
- *Cerium oxide*: These nanoparticles have excellent properties when contrasted with their metal partner. For example, zinc oxide. Iron oxide, silicon dioxide, magnetite, etc.

2.3 Carbon based

The nanoparticles made totally of carbon are known as carbon-based [1, 9, 10]. They can be classified as:

- *Fullerenes*: Fullerenes is a carbon particle that is spherical on shape and made up of carbon molecules held together by sp^2 hybridization. Around 28–1500 carbon atoms form the spherical structure with diameter of 8.2 nm for a single layer and for a multi-layered fullerenes 4–36 nm [9, 10].
- *Graphene*: Graphene is an allotrope of carbon. It is a hexagonal system of honeycomb lattice made of carbon atoms in a 2-D planar surface. The thickness of the graphene is of 1 nm [9, 10].
- *Carbon nano tubes*: In this, nano foil which has a honeycomb lattice of carbon atoms is twisted into a hollow cylinder to frame nanotubes of measurements as low as 0.7 nm [1, 10].
- *Carbon nanofiber*: When graphene nano foil used to produce carbon nanofiber as carbon nanotubes however twisted into a cone or cup shape than a regular cylindrical tube [1, 10].

- *Carbon black*: It is an undefined material comprised of carbon, generally spherical in shape with diameter measurements up to 20–70 nm. Interaction between the particles is high to such an extent that they aggregate and the agglomeration are seen as of 500 nm [1, 10].

3. Nanoparticle as a threat

Nano-technology has acquired an incredible revolution in the industrial division. Due to their exceptional physiochemical and electrical properties, Nano-sized materials have increased a great deal of fascination in the field of hardware, biotechnology and aeronautic design. It is additionally being utilized in the field of medicine NPs similar to the novel delivery system for drugs, DNA and so on. Human is exposed to different non-scale materials since the new developing field of nanotechnology has turned into another danger to human life [11, 12]. The proposed hypothesis is that the NPs of size under 10 nm act similar to gas and can enter human tissues effectively and may abrogate the cell typical biochemical condition [11, 13]. There have been studies on human and murine models that the NPs are exposed through orally they are circulated to the spleen, liver, heart



Figure 3.
The following figure represents usages of nanotechnology/nanoparticles in different field [6].

and lungs also to the brain and gastrointestinal zone, some other exposure routes may incorporate skin, ingestion, inhalation and injection. Some designed NPs are being utilized in many products with direct exposure to people, for instance, ZnO NPs are added to numerous items including cotton texture, Food packaging and rubber for its freshening up and antibacterial attributes, TiO₂ NPs are utilized in food coloring, makeup, skincare item and tattoo pigments, Fe₂O₃ NPs utilized in the final polish on metallic gems (jewelries) [12]. It has been seen that life expectancy of the nanoparticles in human is around 700 days in which it reliably has a risk to the body. Nanoparticles have an incredible risk to human's wellbeing when contrasted with large-sized particles of the similar chemical compound and it is commonly said that toxicities are contrarily corresponding to the size of the nanoparticles [14, 15]. As the utilization of engineered nanoparticles keeps on developing exponentially, an unintended and intended exposure may happen, which will prompt a high level of human wellbeing hazard. End product users, occupationally exposed subjects and the overall population may be in danger of antagonistic impact (**Figure 3**).

The physiochemical properties of NPs impact how they interact with cells and thus, their potential danger. Studies have demonstrated the different properties that make some nanoparticles more toxic than others. Hypothetically, molecules size is likely to add to cytotoxicity. Smaller NPs have a bigger specific surface area and thus in this way increasingly accessible surface area to interact with cell components for example, carbohydrates, protein, nucleic acids and fatty acids. Nanoparticles with small size are liable to enter the cells, causing cellular damage. Some nanoparticles lethality were seen as a function of both size and specific surface area. It has additionally been seen that size of NPs has seen to correspond with reactive oxygen species (ROS) generation when comparing the amount of ROS generation per surface area within certain size range [14–16]. Nanoparticles size between 10 nm or > 30 nm creates comparable level of ROS per surface area. In any case, there was a sensational increment in ROS production per unit surface in particle expanding from 10 to 30 nm. This information or data disclose to us the bits of knowledge with respect to the perplexing connection between NPs properties and Nano toxicity.

4. Toxicity of silver nanoparticles

Silver nanoparticles are progressively utilized in different fields, including health care, medical, food, consumer and industrial purpose because of their novel physical and chemical properties. Because of their unconventional properties, they have been utilized for a few applications, as in medical device coating, drug delivery, health care products, and food industry, as anticancer agent and orthopedics and also as anti-bacterial agents. AgNPs by a long shot the most generally utilized in customers items, for example, in kitchen utensils, toothpaste, bedding, deodorants, nursing bottles, washing machines, nipples and humidifiers [17]. So as to satisfy the necessity of silver NPs different strategy have been utilized for synthesis, conventional technique like chemical and physical strategies have been utilized, yet they are by all accounts expensive and toxic/hazardous [18].

An organic methodology has been utilized in the synthesis of AgNPs utilizing microorganisms, fungi and plant extract prompting to reliable alternative to chemical and physical techniques in acquiring the nanoparticles in controlled particle size. It has been seen that green synthesis of AgNPs with various stabilizing agents, for example, polyethylene glycol, alcohol vinyl, dextran, cyclodextrins and utilizing

“andeli” (rose extract) [19–22]. This expands utilization of AgNPs in different materials has prompted a more straightforward and direct exposure in human and raised potential dangers to health issues. In an in vivo examination (Sprague-Dawley rodents) they were dealt with orally for 28 days with AgNPs in spite of the fact that there was no observable difference in clinical sign and neither in any difference in body or organ weight. The impacts on blood biochemistry have been seen to increase in cholesterol at high doses of AgNP which indicates that hepatotoxicity and increment in alkaline phosphatase. In another investigation, F344 rodents were fed AgNPs for a time period of 90 days and a decrease in body weight in males was seen following a month of exposure and a dose-dependent change was seen in cholesterolemia and alkaline phosphatases activity which proposed that 125 mg/kg body weight of AgNPs may cause liver harm (Figure 4).

It is commonly realized that NPs can be absorbed by the digestive tract not just through the M-cells in the Peyer’s patch yet additionally by numerous organs as shown in Figure 5. Culture of human mesenchymal stem cells incubated with $0.1 \mu\text{g ml}^{-1}$ of normal human lungs fibroblast cells or albumin-capped silver nanoparticles or human glioblastoma cells starch incubated with capped silver nanoparticle (AgNPs) showed genotoxicity up to dosages of $50 \mu\text{g ml}^{-1}$. Albumin-capped AgNPs has been demonstrated to be more genotoxic than polysaccharide-capped AgNPs. Silver nanoparticles exposed to in vivo models like mice were seen to be more toxic than fish to capped AgNPs. However NPs toxicity/genotoxicity was seen as more when the concentration of albumin-capped NPs was increased to $>100 \mu\text{g ml}^{-1}$ (Table 4).

In recent investigation, it has been seen that hepatotoxicity, pulmonary inflammation, genotoxicity, neurotoxicity, inflammatory effects and cytotoxicity have been related with various shape and size of silver nanoparticles. A large number of articles have been proposed that silver nanoparticles assume a noteworthy job in prompting Reactive Oxygen Species (ROS) which in returns lead to cell cytotoxicity and genotoxicity. It has been seen that cytotoxicity has been closely identified to the generation of ROS. For example interaction of AgNPs with mitochondrial can be seen that: 95% of the cell’s energy is generated by mitochondria as it is the powerhouse of the cells. It’s a significant and essential piece of the cell. ROS generation is possible because of the superoxide spillage through the membrane. The interaction of AgNPs with mitochondria which prompts generation of ROS can be clarified in a manner when Ag^+ and silver nanoparticles have a high affinity (–SH) thiol group in cysteine residues. AgNPs disrupts the membrane proteins integrity of the mitochondria, also hampers the membrane permeability of the membrane and abrogate the mitochondrial functions (Figure 6).

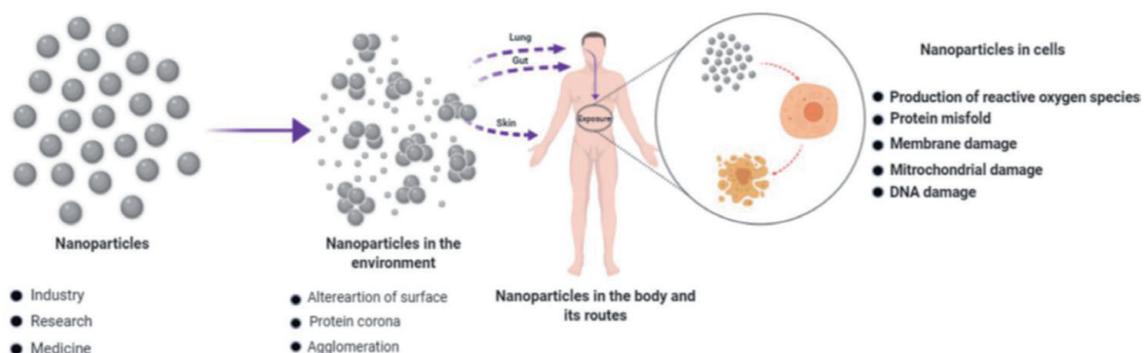


Figure 4.
Nanoparticles pathway and toxicological impact [13, 14].

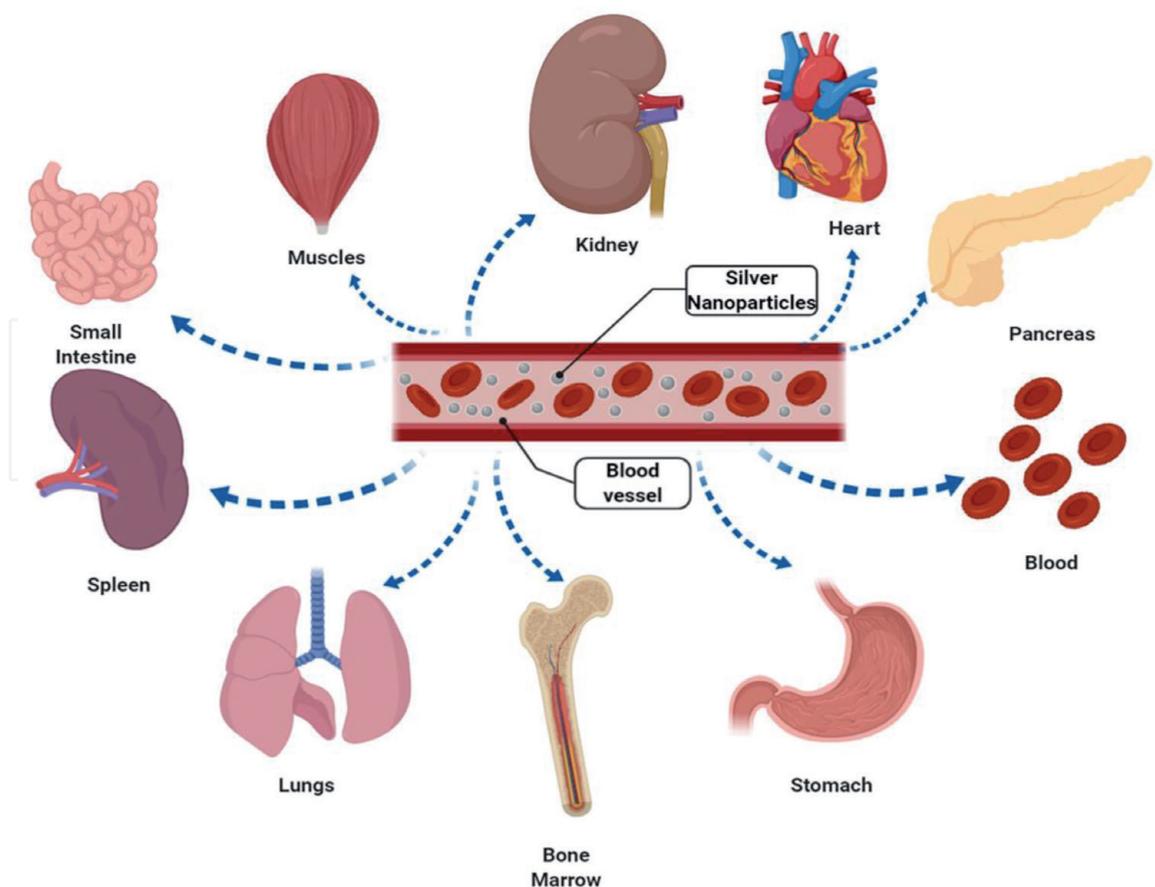


Figure 5.
Main target organ of silver nanoparticles.

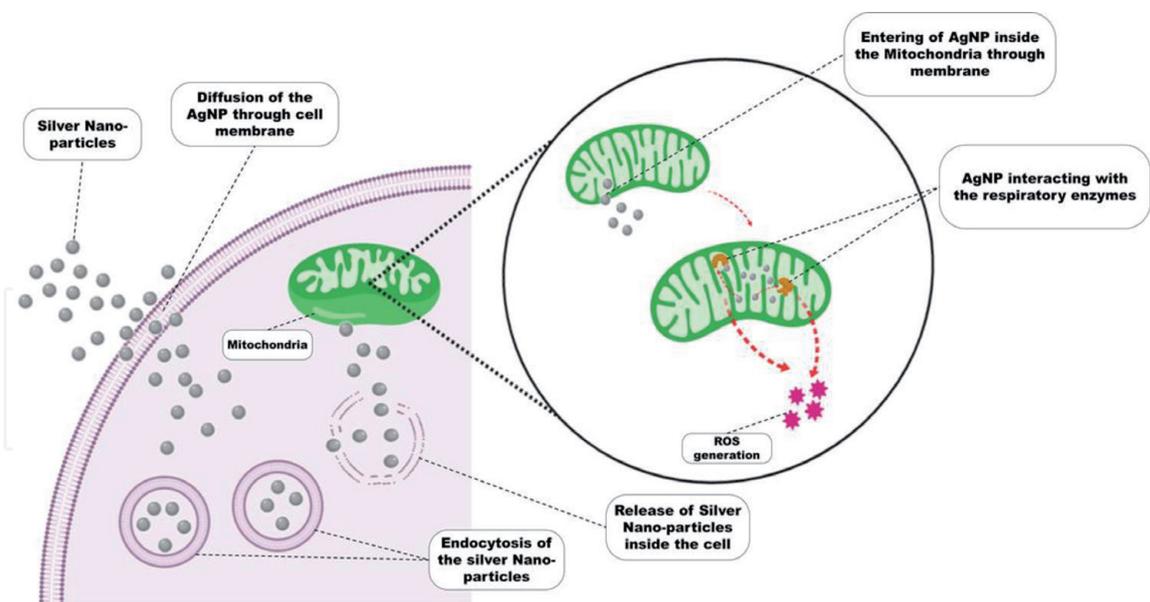


Figure 6.
This figure represents the endogenous ROS production, which are involved in oxidative stress. Interaction between mitochondrial and AgNP generate ROS from mitochondria which leads to cell death [23].

A great deal of studies has demonstrated a connection in ROS generation by silver nanoparticles, oxidative stress and cytotoxicity. Numerous toxicological changes have been reported in embryos when they are exposed to nanoparticles, for example, changes in oxidative stress markers such as apoptosis, changes in expression of genes and lipid oxidation, etc. It has been seen that massive production of free radicals lead to the generation of pro-inflammatory cytokines and furthermore initiation of NOX/

Sr. no.	Classification	Cell	Size (nm)	Cytotoxicity	Genotoxicity
1	Vertebrate/chordata/ mammalian	IMR-90 Human lung fibroblast	6–20	50 $\mu\text{g ml}^{-1}$	Comet assay 5 μm (50 comets were analyzed per concentration) (DNA damage at 50 $\mu\text{g ml}^{-1}$)
2	Vertebrate/chordata/ mammalian, human	A549 Human lung carc.	78	12.5 $\mu\text{g ml}^{-1}$	In this it was indicated that silver NPs mediated ROS-induced genotoxicity
3	Vertebrate/chordata/ mammalian	mES Mouse embryonic stem cells	25	50 $\mu\text{g ml}^{-1}$	50 $\mu\text{g ml}^{-1}$ (this conc. upregulates the DNA damage repair proteins Rad51 and phosphorylated-H2AX expression and even upregulates cell cycle checkpoint protein p53)
4	Vertebrate/chordata/fish	OLHN12 Medaka fish	20–30	1.3 $\mu\text{g ml}^{-1}$	1.2 $\mu\text{g ml}^{-1}$ aneuploidy 15.8%
5	Vertebrate/chordata/ mammalian	BRL 3A Rat liver cells	25	50 $\mu\text{g ml}^{-1}$	10 $\mu\text{g ml}^{-1}$
6	Plantae/liliopsida	<i>Allium cepa</i> 5000 cells	24–55	ROS-induced up to 10 $\mu\text{g ml}^{-1}$ cell death and DNA damage doses 20 $\mu\text{g ml}^{-1}$	No genotoxicity
7	Vertebrate/chordata/fish	Primary trout hepatocytes N = 3, each treatment	35	Significant toxicity from 500–1000 $\mu\text{g ml}^{-1}$	Didn't cause significant lactate dehydrogenase release
8	Vertebrate/chordata/ mammalian	MEF Mouse embryonic fibroblasts	25	50 $\mu\text{g ml}^{-1}$	50 $\mu\text{g ml}^{-1}$ this concentration upregulates the Rad51 and phosphorylates-H2AX expression and also upregulates p53
9	Vertebrates/chordata/ mammalian, human	Human lymphocytes	25–45	Till 400 $\mu\text{g ml}^{-1}$	Till 50 $\mu\text{g ml}^{-1}$ no DNA damage
10	Vertebrate/chordata/ mammalian, human	HepG2 Human hepatocytes 150 cell per group	15–20	13 $\mu\text{g ml}^{-1}$ (58% survival)	Here it was indicated that the AGNPs induces ROS-induced nontoxicity

Table 4.
In vivo cytotoxicity and genotoxicity effects of AgNPs in different organisms [25].

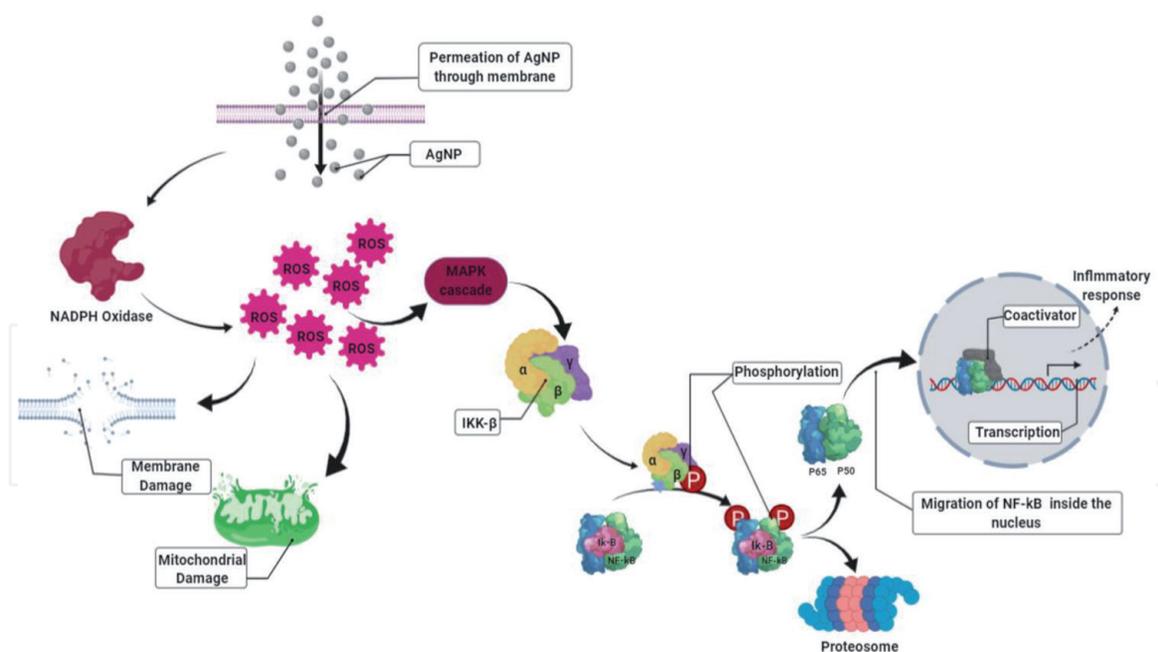


Figure 7. Pictorial representation of activation of cellular mechanisms of inflammatory signal when exposed to AgNP generated from ROS and by strengthening of NADPH oxidase activity. By MAP kinases pathway, activation of oxidative IKK-B which is induced by stress leads to NF-κB translocation and expression of marker and potential mediators of inflammation increases, mitochondrial damage and membrane damage which can cause toxicity in cell and leads to death by apoptosis [24].

NADPH oxidase family. It must be noticed that other than inflammatory effects of oxygen radicals, oxidants helps the release of inflammatory mediators by activating transcription factor including AP-1, hypoxia inducing factor and NF-κB and which prompts to oxidative stress and inflammation. Oxidative stress might have a double role, first as “effector” (by oxidant discharge and induced toxicity) and secondly as “modulator” (managing transcription factor) of chronic micro-inflammation process. This interaction among inflammation and oxidative stress in an amplified manner may prompt to the deleterious impacts brought by silver nanoparticles and which can prompt to DNA damage and cell demise by apoptosis (**Figure 7**).

5. Conclusion

In brief, the ingenious and extensive demand of nanoparticles has led to their extensive production. After use modalities path their way towards exposure to environment as well as to the human health moreover the product which are being used are also in direct contact with the human tissue. Prolong accumulation of the nanoparticle particularly talking about silver nanoparticles.

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