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SILVER NANOPARTICLES (AGNPS): ADVANCES IN SYNTHESIS, CHARACTERIZATION, BIOMEDICAL APPLICATIONS, AND SAFETY CHALLENGES

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ABSTRACT

Silver nanoparticles (AgNPs) have emerged as multifunctional nanomaterials with remarkable physicochemical and biological properties, making them suitable for biomedical, environmental, and industrial applications. AgNPs exhibit potent antimicrobial, antiviral, anti-inflammatory, and anticancer activities that are largely size-, shape-, and surface-dependent. Recent advances in synthesis methodologies, including physical, chemical, and green/biological approaches, enable precise control over particle morphology, surface functionalization, and biocompatibility. Characterization techniques such as TEM, SEM, DLS, XRD, FTIR, and ICP-MS are crucial to correlate physicochemical properties with biological outcomes. Mechanistic studies reveal that AgNPs disrupt microbial membranes, generate reactive oxygen species (ROS), release Ag^+ ions, and interfere with cellular proteins and nucleic acids, resulting in cell death. Biomedical applications include wound healing, drug delivery, antimicrobial coatings, cancer therapy, and diagnostics. Despite the promising therapeutic potential, concerns regarding cytotoxicity, organ accumulation, environmental impact, and regulatory compliance remain critical. Strategies such as green synthesis, surface functionalization, controlled release, and safer-by-design approaches are under investigation to enhance efficacy while minimizing toxicity. This review provides an in-depth discussion of synthesis, characterization, biological mechanisms, biomedical applications, toxicity, regulatory challenges, and future perspectives, highlighting the translational potential of AgNPs.

KEYWORDS: Silver nanoparticles; AgNP; green synthesis; antimicrobial mechanism; wound healing; Nano medicine; toxicity; clinical translation.

1. INTRODUCTION

Nanotechnology has revolutionized biomedical research by enabling the design of materials at the nanoscale with unique physicochemical properties. Among these, **silver nanoparticles (AgNPs)** have emerged as one of the most extensively studied nanomaterials due to their broad-spectrum antimicrobial activity, biocompatibility, and versatility in functionalization.[1,2] AgNPs are particles of silver with sizes typically ranging from 1 to 100 nm, exhibiting high surface area-to-volume ratios, tunable shapes, and surface properties that distinguish them from bulk silver.[3]

Historically, silver has been recognized for its antimicrobial properties for centuries, used in wound care, water purification, and medicinal applications. The advent of nanotechnology has amplified these properties, as the nanoscale form allows for enhanced cellular penetration, controlled ion release, and multiple mechanisms of microbial inhibition. AgNPs exert antimicrobial effects against bacteria, fungi, and viruses through mechanisms including reactive oxygen species (ROS) generation, membrane disruption, and interference with DNA and protein functions.[4]

Beyond antimicrobial applications, AgNPs are being investigated for wound healing, drug delivery, anticancer therapy, diagnostics, and antiviral interventions, highlighting their multidisciplinary relevance in medicine.[5] The design and functionality of AgNPs are

highly influenced by size, shape, surface charge, and capping agents, which dictate their biological interactions, efficacy, and toxicity.

Despite these promising attributes, the translation of AgNPs into clinical practice faces challenges including toxicity, bio distribution, environmental impact, and regulatory hurdles. Understanding the mechanisms of action, optimizing physicochemical properties, and addressing safety concerns are crucial steps toward developing effective and safe AgNP-based therapeutics.[6]

1.1 Advantages and Disadvantages of Silver Nanoparticles

Advantages[7, 8]

1. **Broad-Spectrum Antimicrobial Activity**

AgNPs exhibit potent antibacterial, antifungal, and antiviral properties, effective even against multidrug-resistant pathogens.

2. **Enhanced Surface Area and Reactivity**

The nanoscale size provides a high surface area-to-volume ratio, increasing interactions with microbial membranes and cellular components, enhancing efficacy.

3. **Versatility in Synthesis and Functionalization**

AgNPs can be synthesized via physical, chemical, or green methods and functionalized with polymers, drugs, or targeting ligands for specific biomedical applications.

4. **Multifunctional Applications**

Applications extend beyond antimicrobial use to include wound healing, drug delivery, anticancer therapy, diagnostics, and tissue engineering.

5. **Controlled Release and Targeted Action**

Incorporation into hydrogels, scaffolds, or Nano carriers allows sustained and localized silver ion release, reducing systemic toxicity.

6. **Synergistic Effects**

AgNPs can be combined with antibiotics or other therapeutics to enhance efficacy and overcome microbial resistance.

Table 1: Advantages of Silver Nanoparticles.

Advantages	Description
Broad-Spectrum Antimicrobial Activity	AgNPs exhibit potent antibacterial, antifungal, and antiviral effects, effective even against multidrug-resistant pathogens.
Enhanced Surface Area and Reactivity	The nanoscale size provides a high surface area-to-volume ratio, enhancing interactions with microbial membranes and biomolecules, thereby improving antimicrobial efficacy.
Versatility in Synthesis and Functionalization	AgNPs can be synthesized via physical, chemical, or green methods and functionalized with drugs, polymers, or targeting ligands for specific biomedical purposes.
Multifunctional Applications	AgNPs are utilized in wound healing, drug delivery, anticancer therapy, diagnostics, and tissue engineering due to their adaptable physicochemical and biological properties.
Controlled Release and Targeted Action	Incorporation into hydrogels, scaffolds, or nanocarriers enables sustained and localized silver ion release, minimizing systemic toxicity.
Synergistic Effects	AgNPs enhance the efficacy of antibiotics and other therapeutic agents, helping overcome microbial resistance mechanisms.

Disadvantages[7, 8]

1. **Toxicity and Cytotoxicity**

AgNPs may induce oxidative stress, DNA damage, mitochondrial dysfunction, and inflammation in mammalian cells, particularly at high doses or with small particle sizes.

2. **Biodistribution and Accumulation**

Nanoparticles can accumulate in the liver, kidneys, spleen, and brain, raising concerns about long-term toxicity and organ damage.

3. **Environmental Concerns**

Widespread use may lead to environmental accumulation, affecting microbial ecology and potentially promoting resistance.

4. **Lack of Standardization**

Variability in size, shape, surface chemistry, and synthesis methods leads to inconsistent biological responses and challenges in reproducibility.

5. **Regulatory Challenges**

Nanoparticles face complex regulatory classification, and safety evaluation standards are still evolving, limiting clinical translation.

6. **Cost and Scale-Up Issues**

Large-scale production with reproducible quality, stability, and sterility remains technically challenging and costly.

Table 2: Disadvantages of Silver Nanoparticles.

Disadvantages	Description
Toxicity and Cytotoxicity	AgNPs may cause oxidative stress, DNA damage, mitochondrial dysfunction, and inflammation in mammalian cells, especially at high concentrations or small particle sizes.
Biodistribution and Accumulation	Nanoparticles can accumulate in vital organs (liver, kidney, spleen, brain), raising concerns about chronic toxicity and bioaccumulation.
Environmental Concerns	Excessive use of AgNPs may lead to environmental persistence and disrupt microbial ecosystems, potentially inducing resistance in microorganisms.
Lack of Standardization	Inconsistencies in synthesis, particle size, shape, and surface chemistry lead to variability in biological responses and hinder reproducibility across studies.
Regulatory Challenges	Evolving regulatory frameworks and lack of standardized safety assessment protocols limit AgNP clinical translation and commercialization.
Cost and Scale-Up Issues	Producing AgNPs at industrial scale with consistent quality, stability, and sterility remains technically demanding and economically expensive.

2. SYNTHESIS OF SILVER NANOPARTICLES

Silver nanoparticles (AgNPs) can be synthesized using diverse physical, chemical, and biological methods. The choice of synthetic approach significantly influences nanoparticle size, shape, surface chemistry, stability, and biological activity, which are critical for biomedical and industrial applications. Recent advances have focused on controlling particle morphology, enhancing reproducibility, and developing environmentally friendly processes.[9]

2.1 Physical Methods

Physical methods involve top-down approaches where bulk silver is broken down into nanoparticles using physical energy. Common techniques include evaporation-condensation**,** laser ablation**,** and ball milling**.**[10,11]

a. **Evaporation-Condensation:** Bulk silver is vaporized in a high-temperature furnace, and nanoparticles condense on a cold surface. This method yields high-purity, spherical nanoparticles with narrow size distribution. It avoids chemical contamination, which is advantageous for biomedical applications. However, the method requires high energy consumption**,** precise temperature control, and specialized equipment, making large-scale production challenging.
b. **Laser Ablation:** A high-energy laser is focused on a silver target in liquid or gas phase, causing material ablation and nanoparticle formation. Laser ablation offers excellent control over particle size and shape and produces chemically pure nanoparticles. Parameters such as laser wavelength, pulse duration, and solvent type influence particle morphology. Despite its advantages, laser ablation is limited by low production yield and high operational costs.
c. **Ball Milling:** Mechanical grinding of silver powder in the presence of stabilizers reduces particle size. This is simple and cost-effective but often results in broad size distribution and potential contamination from the milling media.

2.1.1 Advantages of Physical Methods: High purity, chemical-free, reproducible particle formation, and excellent crystallinity.

2.1.2 Limitations: Energy-intensive, expensive equipment, difficult scalability, limited control over narrow particle size at large scale.

Recent studies have explored **hybrid physical approaches**, combining laser ablation with chemical stabilizers or ultra-sonication, to improve size uniformity and stability.

2.2 Chemical Reduction Methods

Chemical reduction is the most widely employed method for synthesizing AgNPs, categorized as a bottom-up approach, where silver ions (Ag^+) are reduced to elemental silver (Ag^0) using reducing agents in solution. The nanoparticles are stabilized using capping agents to prevent aggregation.[12]

A. Common Reducing Agents

a. **Sodium borohydride ($NaBH_4$):** Produces small, uniform nanoparticles (2–20 nm). Rapid reduction can lead to high nucleation rates and small particle size.
b. **Citrate:** Acts as both reducing and capping agent; produces larger spherical nanoparticles with excellent stability.
c. **Ascorbate (Vitamin C):** Mild reducing agent, biocompatible, suitable for biomedical applications.

B. Capping Agents / Stabilizers

Polymers such as polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), and proteins prevent aggregation, enhance colloidal stability, and improve biocompatibility. The choice of stabilizer can modulate surface charge, hydrophilicity, and biological interactions.

Reaction Parameters Affecting Nanoparticle Properties

a. **Temperature:** Higher temperatures accelerate nucleation, producing smaller particles.

b. pH: Influences reduction kinetics and particle morphology.
c. Silver ion concentration: Determines nucleation density and final particle size.

2.2.1 Advantages of Chemical Reduction
a. Scalable and reproducible.
b. Good control over particle size and shape.
c. Compatible with surface functionalization for biomedical applications.

2.2.2 Limitations
a. Potential cytotoxicity from residual reducing agents
b. Environmental concerns due to chemical waste
c. Requires careful purification for clinical use

Recent studies have employed eco-friendly chemical reducers, such as glucose or amino acids, to reduce environmental impact and improve nanoparticle biocompatibility.[13]

2.3 Green / Biological Synthesis
Green synthesis represents a sustainable, environmentally friendly approach using biological entities as reducing and capping agents. Plant extracts, microorganisms (bacteria, fungi, algae), and biomolecules (proteins, polysaccharides) can mediate nanoparticle formation under mild conditions.[14]

A. Plant-Mediated Synthesis
Phytochemicals such as flavonoids, polyphenols, terpenoids, and alkaloids reduce Ag^+ to Ag^0 while simultaneously stabilizing nanoparticles. Reaction parameters such as extract concentration, pH, and temperature influence particle size, shape, and stability. Example: Azadirachta indica leaf extract produces spherical nanoparticles (~10–30 nm) with excellent antimicrobial activity.

B. Microbial Synthesis
Certain bacteria (e.g., Bacillus subtilis, Pseudomonas aeruginosa*)* and fungi *(e.g.,* Fusarium oxysporum*)* secrete enzymes and metabolites that reduce Ag^+ to Ag^0 nanoparticles extracellularly or intracellularly. Advantages include easy scale-up and eco-friendly process, but challenges include slower synthesis rates and risk of contamination.

Biomolecule-Mediated Synthesis
Proteins, peptides, and polysaccharides can act as both reducing and stabilizing agents, producing highly stable AgNPs suitable for biomedical applications.

2.3.1 Advantages of Green Synthesis
a. Eco-friendly and sustainable
b. Biocompatible nanoparticles
c. Mild reaction conditions, reducing energy and chemical use

2.3.2 Limitations
a. Batch-to-batch variability
b. Polydispersity
c. Limited control over particle morphology at large scale

Recent research focuses on **hybrid green-chemical methods**, where low-toxicity chemical reducers are combined with plant extracts to enhance reproducibility while maintaining biocompatibility.[15]

Table 3: Physical, chemical, and biological methods.

Method	Mechanism	Advantages	Limitations
Physical	Top-down; mechanical or laser energy breaks bulk silver	High purity, chemical-free	Energy-intensive, costly, scale-up difficult
Chemical	Bottom-up; Ag^+ reduced by chemical agents, stabilized by polymers	Scalable, tunable size/shape	Residual toxicity, environmental concerns
Green/Biological	Reduction using plant extracts, microbes, or biomolecules	Eco-friendly, biocompatible	Polydispersity, reproducibility, slower synthesis

3. CHARACTERIZATION TECHNIQUES OF SILVER NANOPARTICLES (AGNPS)

Characterization of silver nanoparticles (Ag-NPs) is a critical step in nanoscience research because the biological activity, stability, and safety profile of Ag-NPs are strongly influenced by their physicochemical properties such as size, shape, surface charge, crystallinity, and surface functionalization. Comprehensive characterization ensures reproducibility, guides synthesis optimization, and provides insights into potential biomedical applications. Several analytical techniques are routinely employed for Ag-NP characterization, spanning optical, microscopic, spectroscopic, and physicochemical approaches.[16,17,18,19]

3.1 Optical Characterization

3.1.1 UV–Visible (UV–Vis) Spectroscopy
UV–Vis spectroscopy is a rapid and non-destructive method to confirm the formation of AgNPs. Silver nanoparticles exhibit surface plasmon resonance (SPR)**,** a collective oscillation of conduction electrons upon interaction with light, typically producing an absorption peak between 400–450 nm depending on particle size and shape.

Applications
a. Confirming nanoparticle formation
b. Monitoring reaction kinetics
c. Estimating particle size qualitatively

Advantages: Fast, simple, and cost-effective.
Limitations: Cannot provide exact size or shape; complementary techniques required for detailed characterization.

3.2 Microscopic Techniques

3.2.1 Transmission Electron Microscopy (TEM)

TEM provides high-resolution images, allowing direct visualization of particle size, shape, and aggregation state**.** TEM can resolve features at the nanometer and sub-nanometer scale.
Applications: Determining particle morphology (spherical, triangular, rod-shaped), size distribution, and lattice structure
Advantages: High spatial resolution; allows crystalline structure analysis when combined with selected area electron diffraction (SAED)
Limitations: Sample preparation can induce artifacts; small field of view; expensive equipment.

3.2.2 Scanning Electron Microscopy (SEM)

SEM offers surface morphology analysis with slightly lower resolution than TEM but is widely used to study nanoparticle aggregation, surface topography, and coatings on substrates.
Advantages: Easier sample preparation than TEM; can visualize surface interactions
Limitations: Limited to surface analysis; requires conductive coating for non-conductive samples; lower resolution than TEM.

3.3 Dynamic Light Scattering (DLS) and Zeta Potential

3.3.1 DLS

DLS measures the **hydrodynamic diameter** of nanoparticles dispersed in a liquid medium, providing particle size distribution and polydispersity index (PDI).
Applications: Monitoring colloidal stability; evaluating aggregation tendencies.
Advantages: Rapid and non-destructive; suitable for colloidal suspensions.
Limitations: Sensitive to impurities; provides hydrodynamic size rather than core size.

3.3.2 Zeta Potential

Zeta potential measures the **surface charge** of nanoparticles, reflecting colloidal stability. High absolute zeta potential ($\geq \pm 30$ mV) indicates strong electrostatic repulsion and stable suspensions.
Applications: Predicting stability, formulation optimization, surface modification studies
Advantages: Quick and quantitative assessment of stability
Limitations: Influenced by pH, ionic strength, and medium composition.

3.4 Structural and Crystallinity Analysis

3.4.1 X-ray Diffraction (XRD)

XRD identifies the crystalline nature of Ag-NPs and provides information on crystal structure, lattice parameters, and average crystallite size (using the Scherrer equation). Peaks corresponding to face-centered cubic (fcc) silver are typically observed at $2\theta \approx 38°$, 44°, 64°, and 77°.
Advantages: Confirms crystallinity; non-destructive
Limitations: Provides bulk average information; cannot resolve individual nanoparticle morphology

3.5 Surface Chemistry Analysis

3.5.1 Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR identifies functional groups present on nanoparticle surfaces, which originate from capping agents or biological molecules in green synthesis. Characteristic bands indicate interactions between silver and biomolecules (e.g., O–H, N–H, C=O).
Applications: Understanding surface functionalization and stabilization
Advantages: Simple, fast, non-destructive
Limitations: Limited spatial resolution; cannot provide particle size.

3.5.2 X-ray Photoelectron Spectroscopy (XPS)

XPS provides elemental composition and chemical states on the nanoparticle surface. It can distinguish between metallic silver (Ag^0) and ionic silver (Ag^+), essential for understanding reactivity and toxicity.
Advantages: Quantitative surface elemental analysis; chemical state determination
Limitations: Surface-sensitive; requires ultra-high vacuum and expensive instrumentation.

3.6 Quantitative Analysis

3.6.1 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS accurately measures **total silver content** and released Ag^+ ions in biological and environmental samples. It is essential for **toxicity evaluation** and dosage standardization.
Advantages: Highly sensitive (ppb levels); quantitative
Limitations: Destructive; requires sample digestion; cannot distinguish nanoparticle vs. ionic forms alone.

3.6.2 Atomic Absorption Spectroscopy (AAS)

AAS is also used to determine total silver concentration in solution or biological tissues.
Advantages: Simple and widely available
Limitations: Less sensitive than ICP-MS; cannot analyze particle size.

3.7 Complementary Techniques

a. **Thermogravimetric Analysis (TGA):** Evaluates thermal stability and amount of capping agents on nanoparticle surfaces.
b. **Differential Scanning Calorimetry (DSC):** Provides thermal transitions and nanoparticle stability information.
c. **Scanning Transmission Electron Microscopy (STEM):** Combines SEM and TEM capabilities for

high-resolution imaging and compositional mapping.

3.8 Importance of Multi-Technique Characterization

A single technique is insufficient to fully characterize Ag-NPs. For example, TEM provides size and shape, but not colloidal stability; DLS gives hydrodynamic size, but not morphology. Therefore, a combination of UV–Vis, TEM/SEM, DLS, zeta potential, XRD, FTIR/XPS, and ICP-MS is recommended for comprehensive analysis.[19, 20]

Table 4: Multi-Technique Characterization.

Technique	Property Measured	Advantages	Limitations
UV–Vis	Formation, SPR	Rapid, simple	Cannot determine exact size/shape
TEM	Size, shape, aggregation	High resolution	Small field, expensive
SEM	Surface morphology	Easy sample prep	Lower resolution than TEM
DLS	Hydrodynamic size, PDI	Fast, colloidal analysis	Sensitive to impurities
Zeta Potential	Surface charge, stability	Quantitative	Medium-dependent
XRD	Crystallinity	Confirms lattice structure	Bulk average info
FTIR	Surface functional groups	Simple, non-destructive	No particle size info
XPS	Surface composition, oxidation state	Quantitative, chemical info	Surface-sensitive
ICP-MS / AAS	Total silver content	Highly sensitive	Destructive

4. PHYSICOCHEMICAL PARAMETERS INFLUENCING BIOLOGICAL ACTIVITY

The biological activity of silver nanoparticles (AgNPs) is highly dependent on their intrinsic physicochemical properties. Parameters such as size, shape, surface charge, crystallinity, and functionalization critically determine their interaction with biological systems, thereby influencing their antimicrobial potency, cytotoxicity, biodistribution, and overall therapeutic potential.[21,22]

4.1. Particle Size

Size plays a pivotal role in dictating nanoparticle reactivity and cellular uptake. Smaller AgNPs (<10 nm) exhibit enhanced antimicrobial activity due to their high surface area-to-volume ratio and greater ion release potential, facilitating stronger interactions with bacterial membranes and intracellular components. However, ultrasmall particles also tend to induce higher cytotoxicity by generating reactive oxygen species (ROS) and penetrating organelles such as mitochondria and nuclei.

4.2. Particle Shape

The morphology of AgNPs significantly affects their biological interactions. Studies have shown that triangular or truncated particles display superior antimicrobial properties compared to spherical or rod-shaped counterparts, attributed to the presence of high-energy facets such as {111} planes. Shape-dependent cellular internalization has also been reported, with spherical particles being more readily endocytosed compared to rods.

4.3. Surface Charge (Zeta Potential)

Surface charge governs colloidal stability and interactions with negatively charged biological membranes. Positively charged AgNPs exhibit enhanced adherence to bacterial cell walls and mammalian cell membranes, leading to greater antimicrobial activity but also increased cytotoxicity.[7] Neutral or negatively charged particles, often stabilized by polymers, show reduced toxicity and longer circulation times in vivo.

4.4. Crystallinity and Defects

The crystalline structure and defect density of AgNPs modulate their dissolution rate and ion release. Higher crystallinity enhances structural stability, whereas defect-rich particles promote faster Ag+ release, contributing to antimicrobial efficacy but also potential toxicity.

4.5. Surface Functionalization

Surface coatings and functional groups significantly alter the biological identity of AgNPs. Functionalization with biocompatible polymers (e.g., polyethylene glycol, chitosan) improves stability, reduces nonspecific protein adsorption, and lowers cytotoxicity. Conversely, conjugation with targeting ligands or biomolecules enhances specificity for drug delivery or imaging applications.

4.6. Aggregation State

Nanoparticle aggregation reduces effective surface area, ion release, and bioactivity. Stable, monodispersed formulations are therefore preferred for biomedical applications. Environmental factors such as ionic strength, pH, and protein corona formation influence aggregation tendencies in physiological conditions.[22]

4.7 Dose and Exposure Time

Finally, the biological activity of AgNPs is dose- and time-dependent. Low concentrations may provide antimicrobial or therapeutic benefits, whereas prolonged exposure or higher doses increase the likelihood of cytotoxic and genotoxic effects.[23]

Table: - 5 Physicochemical parameters of AgNPs and their influence on biological activity.

Parameter	Description/Variation	Impact on Biological Activity
Size	Small (<10 nm) vs. larger (>50 nm)	Smaller particles release more Ag^+ ions, penetrate membranes easily, and show stronger antimicrobial activity but higher cytotoxicity
Shape	Spherical, rod, triangular, truncated	Triangular/truncated particles exhibit higher antimicrobial activity due to {111} facets; spherical particles show higher uptake
Surface Charge (Zeta Potential)	Positive, neutral, negative	Positive charge enhances bacterial binding but increases cytotoxicity; neutral/negative charge improves circulation and reduces toxicity
Crystallinity/Defects	High vs. low crystallinity, defect-rich surfaces	Defect-rich particles dissolve faster, releasing more Ag^+ ions; crystalline particles show higher stability
Surface Functionalization	Polymer coating (PEG, chitosan), biomolecule conjugation	Biocompatible coatings reduce aggregation, toxicity, and immune recognition; functionalization improves targeting
Aggregation State	Monodispersed vs. aggregated	Aggregation reduces surface area, ion release, and antimicrobial activity; stability critical in physiological conditions
Dose & Exposure Time	Low vs. high concentration; short vs. prolonged exposure	Low doses show therapeutic benefit; high/prolonged exposure induces oxidative stress, cytotoxicity, and genotoxicity

5. MECHANISMS OF BIOLOGICAL ACTION

Silver nanoparticles (AgNPs) exert their biological effects through multiple, often interconnected, mechanisms. These mechanisms are primarily governed by their physicochemical properties, environmental factors, and interactions with biological components. While the antimicrobial properties of AgNPs are well-documented, recent studies have expanded their biological action profile to include anticancer, anti-inflammatory, and wound-healing effects.[24,25,26]

5.1. Ag^+ Ion Release

One of the most significant contributors to AgNP bioactivity is the release of silver ions (Ag^+). Dissolved Ag^+ interacts with thiol groups in bacterial enzymes and proteins, disrupting metabolic pathways and inducing cell death. In mammalian systems, Ag^+ may modulate signaling cascades and induce oxidative stress, contributing to both therapeutic and toxicological outcomes.

5.2. Reactive Oxygen Species (ROS) Generation

AgNPs catalyze the generation of reactive oxygen species, including superoxide radicals, hydroxyl radicals, and hydrogen peroxide. Elevated ROS disrupt lipid membranes, oxidize proteins, and damage DNA, leading to apoptosis or necrosis in microbial and mammalian cells. Controlled ROS generation underpins the potential anticancer effects of AgNPs by selectively inducing tumor cell apoptosis.

5.3. Membrane Disruption

Direct interaction between AgNPs and lipid bilayers compromises membrane integrity. Nanoparticles embed in the phospholipid matrix, leading to increased permeability, leakage of cellular contents, and loss of membrane potential. This mechanism is particularly important in bacterial killing and biofilm inhibition.

5.4. Protein and DNA Interaction

AgNPs and released Ag^+ bind to nucleic acids and ribosomal subunits, impairing transcription, translation, and replication processes. DNA damage, including strand breaks and cross-linking, has been reported in both microbial and mammalian cells, contributing to cytotoxicity and genotoxicity.[26]

5.5. Mitochondrial Dysfunction

In mammalian cells, AgNPs penetrate mitochondria, disrupting the electron transport chain and enhancing ROS production. This leads to a loss of ATP production and activation of intrinsic apoptotic pathways. Such mechanisms are exploited in anticancer strategies but raise concerns for systemic toxicity.

5.6. Immunomodulatory Effects

AgNPs influence immune responses by modulating cytokine production, macrophage activation, and inflammatory pathways. While low concentrations may enhance wound healing and anti-inflammatory effects, higher doses provoke pro-inflammatory and immunotoxic outcomes.

5.7. Biofilm Inhibition

AgNPs prevent biofilm formation by interfering with quorum sensing and disrupting extracellular polymeric substances. This mechanism is particularly valuable in overcoming multidrug resistance in pathogens.

6. BIOMEDICAL APPLICATIONS

Silver nanoparticles (AgNPs) have emerged as versatile agents in biomedical sciences due to their broad-spectrum antimicrobial activity, biocompatibility, and

ability to be functionalized with therapeutic or diagnostic agents. Their applications extend across infectious disease control, wound management, drug delivery, imaging, and cancer therapy, with several products already approved for clinical use.[27, 28,29]

6.1. Antimicrobial Agents

The most widely recognized application of AgNPs is in antimicrobial therapy. AgNPs are effective against Gram-positive and Gram-negative bacteria, fungi, and some viruses. Their multi-targeted mechanisms, including membrane disruption, ROS generation, and protein/DNA interactions, make them particularly valuable in addressing antimicrobial resistance. AgNPs are incorporated into medical devices such as catheters, implants, and surgical instruments to prevent nosocomial infections.

6.2. Wound Healing and Tissue Regeneration

AgNP-based dressings promote wound healing by preventing infection, reducing inflammation, and enhancing re-epithelialization. Commercial products such as *Acticoat*™ and *SilvaSorb*™ are already used in burn and chronic wound management. In addition to antimicrobial protection, AgNPs modulate cytokine production and angiogenesis, thereby accelerating tissue regeneration.

6.3. Drug Delivery Systems

AgNPs serve as nanocarriers for targeted drug delivery, benefiting from their high surface area, tunable size, and ease of functionalization. Conjugation with antibiotics, anticancer agents, or biomolecules improves drug stability, enhances site-specific delivery, and minimizes off-target effects. Stimuli-responsive AgNPs (pH- or light-sensitive) are being developed for controlled release applications.

6.4. Cancer Therapy

AgNPs demonstrate intrinsic anticancer properties by inducing oxidative stress, DNA damage, and apoptosis in tumor cells. When functionalized with ligands or chemotherapeutic agents, they enhance targeted therapy and reduce systemic toxicity. Their ability to synergize with conventional chemotherapy and radiotherapy makes them promising adjunctive agents.

6.5. Diagnostic and Imaging Applications

Due to their unique optical and surface plasmon resonance properties, AgNPs are used in biosensing, bioimaging, and diagnostic platforms. They enhance contrast in surface-enhanced Raman scattering (SERS), fluorescence imaging, and molecular diagnostics. AgNP-based sensors are under development for rapid detection of pathogens, biomarkers, and toxins.

6.6. Antiviral Applications

AgNPs show activity against viruses such as HIV, influenza, and SARS-CoV-2 by binding to viral surface proteins, blocking host cell entry, and disrupting replication. These properties are being explored in coatings for personal protective equipment (PPE) and antiviral formulations.

6.7. Dental and Orthopedic Applications

AgNPs are incorporated into dental composites, restorative materials, and bone cements to prevent bacterial colonization and improve long-term implant success. Orthopedic implants with AgNP coatings reduce infection risks while supporting Osseo integration.

Table: -6 Biomedical applications of silver nanoparticles (AgNPs) and their outcomes.

Application Area	Mode of Action / Role	Examples / Products	Status
Antimicrobial agents	Disrupt membranes, generate ROS, inhibit enzymes/DNA	AgNP-coated catheters, implants	Clinical/Approved
Wound healing	Prevent infection, reduce inflammation, promote angiogenesis	*Acticoat*™, *SilvaSorb*™ dressings	Clinical/Approved
Drug delivery	Nanocarriers for antibiotics, anticancer drugs, biomolecules	AgNP–antibiotic conjugates	Preclinical/Clinical trials
Cancer therapy	Induce apoptosis, enhance chemo-/radiotherapy	AgNP-based anticancer formulations	Preclinical/Trials
Diagnostics & Imaging	SERS enhancement, fluorescence, biosensors	AgNP-based diagnostic kits	Preclinical/Prototype
Antiviral therapy	Inhibit viral entry, replication	AgNP-coated PPE, antiviral gels	Experimental/Trials
Dental applications	Antibacterial composites, restorative materials	AgNP-enhanced dental fillings	Clinical/Preclinical
Orthopedic implants	Antimicrobial coatings, infection prevention	AgNP-coated bone cements	Preclinical/Trials

7. TOXICITY, BIODISTRIBUTION, AND MECHANISMS OF TOXICITY

The clinical translation of silver nanoparticles (AgNPs) is significantly influenced by their safety profile. While AgNPs offer promising biomedical applications, concerns about toxicity, bio distribution, and long-term fate remain critical barriers to their widespread use.

7.1 Bio-distribution

Following administration, AgNPs undergo systemic distribution depending on particle size, shape, surface charge, and route of exposure. Studies in rodents have shown that intravenously administered AgNPs preferentially accumulate in the liver, spleen, kidney, lungs, and brain, largely due to uptake by the reticuloendothelial system (RES). Smaller particles (<10 nm) exhibit higher tissue penetration and may cross the blood–brain barrier (BBB), raising concerns about potential neurotoxicity. Surface functionalization with polyethylene glycol (PEG) or proteins has been demonstrated to prolong circulation time and reduce rapid clearance.

7.2 Toxicity Mechanisms

Several mechanisms underlie AgNP-induced cytotoxicity and tissue injury:

1. **Oxidative Stress and ROS Generation:** AgNPs release silver ions (Ag^+), which catalyze the production of reactive oxygen species (ROS). Elevated ROS levels cause lipid peroxidation, mitochondrial dysfunction, protein denaturation, and DNA strand breaks.
2. **Mitochondrial Dysfunction and Apoptosis:** AgNPs disrupt mitochondrial membrane potential, triggering intrinsic apoptotic pathways involving cytochrome c release and caspase activation.
3. **Inflammatory Responses:** AgNPs stimulate pro-inflammatory cytokine release (IL-6, TNF-α, IL-1β), leading to chronic inflammation and potential tissue damage.
4. **Geno toxicity and DNA Damage:** Both silver ions and nanoparticles may interact with DNA, inducing mutations, chromosomal aberrations, and impaired repair mechanisms.
5. **Protein Corona and Immunological Effects:** In biological fluids, AgNPs rapidly adsorb proteins to form a "protein corona," influencing immune recognition. Some studies suggest altered immune responses, complement activation, and hypersensitivity reactions.

7.3 Organ-Specific Toxicity

Liver: Hepatocellular vacuolation, necrosis, and altered enzyme activity have been reported following prolonged exposure.

Kidneys: AgNPs accumulate in renal tissue, impairing glomerular filtration and tubular function.

Lungs: Inhaled AgNPs induce alveolar inflammation, fibrosis, and epithelial damage.

Central Nervous System (CNS): Nanoparticles crossing the BBB may impair neurotransmitter function, synaptic signaling, and neuronal viability.

7.4 Risk Mitigation Strategies

Efforts to minimize AgNP toxicity include:

a. Surface modifications (PEGylation, biomolecule conjugation) to reduce ion release and nonspecific tissue uptake.
b. Controlled size synthesis to balance therapeutic efficacy and minimize toxicity.
c. Developing biodegradable or stimuli-responsive AgNP formulations to improve clearance.

Overall, while AgNPs show immense therapeutic promise, their toxicity and biodistribution must be carefully evaluated using standardized in vitro and in vivo models, alongside long-term pharmacokinetic and toxicological studies.

8. CLINICAL TRANSLATION AND REGULATORY LANDSCAPE

The clinical application of silver nanoparticles (AgNPs) has attracted substantial interest owing to their potent antimicrobial and wound-healing properties. Despite extensive preclinical evidence, translation into clinical use has been cautious due to safety, reproducibility, and regulatory concerns.[30,31]

8.1 Current Clinical Applications

Several silver-based products are already in clinical use, primarily in wound management and medical devices. Silver-impregnated dressings (e.g., Acticoat™, SilvaSorb™) are widely used for burn and chronic wound care, demonstrating accelerated healing and reduced infection rates. Catheters, endotracheal tubes, and orthopedic implants coated with silver have also shown reduced microbial colonization in clinical settings. Moreover, AgNP-based topical formulations are undergoing clinical evaluation for their efficacy in dermatological and dental applications.

8.2 Barriers to Clinical Translation

a. Safety Concerns: Long-term toxicity, biodistribution, and nanoparticle clearance remain incompletely understood.
b. Standardization Challenges: Variability in synthesis methods, particle size, and surface chemistry lead to inconsistent biological responses.
c. Regulatory Complexity: Unlike conventional drugs, nanomaterials face ambiguity in regulatory classification (drug, device, or combination product).
d. Scale-up and Manufacturing: Industrial-scale synthesis with reproducible quality, stability, and sterility poses significant challenges.

8.3 Regulatory Framework

a. United States (FDA): The FDA evaluates AgNP-based products on a case-by-case basis, depending on intended use. Silver dressings are regulated as medical devices, while systemic formulations may require Investigational New Drug (IND) approval.
b. European Union (EMA): AgNP-containing products are reviewed under the European Medicines Agency and Medical Device Regulation (MDR), with emphasis on safety, biocompatibility, and risk–benefit assessment.
c. Other Jurisdictions: Countries such as Canada, Japan, and India follow similar hybrid frameworks,

with growing emphasis on Nano toxicology data and Good Manufacturing Practice (GMP) compliance.

8.4 Future Directions for Regulatory Acceptance

a. Development of standardized characterization protocols (size, charge, purity, ion release).
b. Establishing long-term safety databases for chronic exposure studies.
c. Harmonization of global regulations to streamline clinical trial approval.
d. Advancing biodegradable and stimuli-responsive AgNP formulations that reduce systemic burden and improve clearance.

8.5 Clinical Translation Outlook

While silver-based products already occupy a niche in wound care and implant coatings, systemic applications such as intravenous formulations, cancer therapeutics, and antiviral agents require more rigorous safety evaluations. A close collaboration between researchers, industry, and regulatory agencies is critical to bridge the gap between laboratory innovation and patient-ready Nano medicines.[32]

9. STRATEGIES TO IMPROVE SAFETY AND EFFICACY

The translation of silver nanoparticles (AgNPs) into clinical use requires careful optimization of their safety and therapeutic performance. Several strategies have been developed to mitigate toxicity, enhance biocompatibility, and maximize biomedical benefits.[33,34]

9.1. Surface Functionalization and Coating

Surface modification of AgNPs with biocompatible polymers such as polyethylene glycol (PEG), chitosan, or polyvinylpyrrolidone (PVP) reduces aggregation, minimizes opsonization, and improves circulation half-life. PEGylation also prevents nonspecific protein adsorption, thereby lowering immunogenicity and enhancing therapeutic outcomes. Biomolecule conjugation (e.g., with peptides, antibodies, or aptamers) provides targeted delivery, improving efficacy while minimizing off-target effects.

9.2. Controlled Size and Shape Engineering

Tuning nanoparticle size and morphology directly influences biological activity and toxicity. Smaller particles (<10 nm) demonstrate higher cellular penetration but greater potential for toxicity, whereas particles in the 20–50 nm range offer a favorable balance between antimicrobial efficacy and reduced systemic burden. Spherical nanoparticles are generally less cytotoxic than sharp-edged triangular or rod-shaped forms due to lower oxidative stress induction.

9.3. Green and Biogenic Synthesis

Green synthesis using plant extracts, microorganisms, and biopolymers produces AgNPs with natural capping agents that enhance stability and biocompatibility. These eco-friendly approaches generate particles with reduced ion leaching and controlled release, lowering cytotoxic risks. Furthermore, biogenic capping often imparts additional therapeutic benefits, such as antioxidant or anti-inflammatory activity.[35]

9.4. Controlled Release Systems

Incorporation of AgNPs into hydrogels, liposomes, nanofibers, and polymeric scaffolds enables sustained and localized release of silver ions. This reduces systemic toxicity and ensures prolonged antimicrobial action at the site of infection or injury. Smart delivery platforms responsive to pH, enzymes, or temperature further improve site-specific action.[36]

9.5. Combination Therapy

Co-delivery of AgNPs with conventional antibiotics, antifungals, or anticancer drugs enhances therapeutic efficacy via synergistic mechanisms while reducing the required dose of both agents. This approach also helps overcome drug resistance and limits silver-associated toxicity.

9.6. Regulatory and Standardization Approaches

Standardizing characterization (size, charge, surface chemistry, ion release) and adopting Good Manufacturing Practices (GMP) are crucial to ensuring reproducibility and safety. Regulatory-guided frameworks for preclinical evaluation and long-term toxicity studies provide essential data for clinical translation.

10. KNOWLEDGE GAPS AND FUTURE DIRECTIONS

Despite significant progress in understanding silver nanoparticles (AgNPs), several knowledge gaps hinder their full clinical translation. Addressing these issues will require interdisciplinary collaboration between chemists, toxicologists, biomedical researchers, and regulatory authorities.[37,38]

10.1. Long-Term Toxicity and Bio distribution

Most toxicity studies on AgNPs focus on acute or short-term exposures. The long-term fate, accumulation, and clearance of nanoparticles in vital organs such as the liver, kidneys, and brain remain insufficiently understood. Chronic exposure studies with standardized protocols are needed to assess cumulative effects and potential delayed toxicity.

10.2. Mechanistic Understanding of Biological Interactions

Although oxidative stress, ion release, and protein corona formation have been identified as major contributors to toxicity, the exact molecular pathways underlying AgNP-induced genotoxicity, immunomodulation, and neurotoxicity require further clarification. Advanced omics approaches (proteomics, metabolomics, and transcriptomics) could provide deeper mechanistic insights.

10.3. Standardization and Reproducibility

Variability in synthesis methods, particle size, surface chemistry, and functionalization leads to inconsistent biological outcomes. Establishing international standards for AgNP characterization, including ion release kinetics and surface modifications, is essential for reproducibility and comparability across studies.

10.4. Translational and Clinical Data Gaps

While AgNP-based wound dressings and coatings are clinically approved, robust randomized controlled trials (RCTs) for systemic applications (e.g., cancer therapy, antiviral therapy) are lacking. More clinical data on efficacy, safety, and pharmacokinetics will be crucial for regulatory approval.

10.5. Environmental and Ecotoxicological Impacts

Large-scale use of AgNPs in medical devices, consumer products, and textiles raises concerns about environmental accumulation and microbial resistance. Research into sustainable disposal, degradation, and environmental interactions of AgNPs is urgently needed.

10.6. Integration with Emerging Technologies

Future research should explore integrating AgNPs with smart drug delivery systems**,** biodegradable carriers**,** and nanocomposite scaffolds to improve site-specific action and reduce systemic burden. Synergistic combinations with other nanomaterials (e.g., graphene, gold, liposomes) may unlock new biomedical applications.

10.7 Future Perspectives

Moving forward, AgNP research must shift from descriptive studies to standardized, mechanism-driven, and clinically oriented investigations**.** A stronger focus on patient-centered applications, regulatory harmonization, and environmental sustainability will be vital to ensure safe and effective translation. Collaborative efforts between academia, industry, and government agencies will accelerate this process, ultimately bridging the gap between laboratory innovation and clinical practice.[39]

11. CONCLUSIONS

Silver nanoparticles (AgNPs) represent a rapidly advancing class of nanomaterials with immense biomedical potential due to their potent antimicrobial, wound-healing, diagnostic, and therapeutic properties. Advances in synthesis and characterization techniques have enabled the design of AgNPs with tunable physicochemical properties, thereby enhancing their safety and efficacy profiles. Nevertheless, significant challenges remain, particularly regarding toxicity, bio distribution, and regulatory standardization.

While AgNPs have already found successful applications in wound dressings, coatings for medical devices, and topical formulations, broader systemic applications such as anticancer and antiviral therapies require more rigorous evaluation. The balance between therapeutic benefits and potential risks is a central determinant of their clinical translation.

Future progress will depend on addressing key knowledge gaps—particularly in long-term toxicity, standardization, and environmental safety—through collaborative, multidisciplinary research. Integration with emerging technologies such as smart delivery systems and biodegradable carriers offers promising avenues to improve specificity and minimize systemic burden.

Overall, AgNPs stand at the intersection of innovation and clinical utility. With careful optimization of their design, regulatory compliance, and translational pathways, these versatile nanomaterials have the potential to transform next-generation medicine and contribute significantly to global healthcare.

12. REFERENCES

1. Rai M, et al. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv*, 2009; 27(1): 76-83.
2. Franci G, et al. Silver nanoparticles as potential antibacterial agents. *Molecules*, 2015; 20(5): 8856-74.
3. Zhang XF, et al. Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. *Int J Mol Sci*, 2016; 17(9): 1534.
4. Kim S, et al. Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. *Toxicol In Vitro*, 2009; 23(6): 1076-84.
5. AshaRani PV, et al. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano*, 2009; 3(2): 279-90.
6. Chaloupka K, et al. Nanosilver as a new generation of nanoproduct in biomedical applications. *Trends Biotechnol*, 2010; 28(11): 580-8.
7. Torchilin VP. Multifunctional and stimuli-sensitive pharmaceutical nanocarriers. *Eur J Pharm Biopharm*, 2009; 71(3): 431-44.
8. Benn TM, Westerhoff P. Nanoparticle silver released into water from commercially available sock fabrics. *Environ Sci Technol*, 2008; 42(11): 4133-9.
9. Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B. Synthesis of silver nanoparticles: chemical, physical and biological methods. *Res Pharm Sci*, 2014; 9(6): 385–406.
10. Bae CH, Nam SH, Park SM. Formation of silver nanoparticles by laser ablation of a silver target in NaCl solution. *Appl Surf Sci*, 2002; 197–198: 628–634.
11. Henglein A. Physicochemical properties of small metal particles in solution: "microelectrode" reactions, chemisorption, composite metal particles, and the atom-to-metal transition. *J Phys Chem*, 1993; 97(21): 5457–5471.

12. Amendola V, Polizzi S, Meneghetti M. Laser ablation synthesis of gold nanoparticles in organic solvents. *J Phys Chem B*, 2006; 110(14): 7232–7237.
13. Natsuki J, Natsuki T, Hashimoto Y. A review of silver nanoparticles: Synthesis methods, properties and applications. *Int J Mater Sci Appl*, 2015; 4(5): 325–332.
14. Jain P, Pradeep T. Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. *Biotechnol Bioeng*, 2005; 90(1): 59–63.
15. Banerjee P, Satapathy M, Mukhopahayay A, Das P. Leaf extract mediated green synthesis of silver nanoparticles. *J Bionanosci*, 2014; 8(3): 205–213.
16. Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: Green synthesis and their antimicrobial activities. *Adv Colloid Interface Sci*, 2009; 145(1–2): 83–96.
17. Paramelle D, Sadovoy A, Gorelik S, et al. A rapid method to estimate the concentration of citrate capped silver nanoparticles. *Colloids Surf B Biointerfaces*, 2014; 123: 620–626.
18. Amendola V, Meneghetti M. Laser ablation synthesis in solution and size manipulation of noble metal nanoparticles. *Phys Chem Chem Phys*, 2009; 11(20): 3805–3821.
19. Mittal AK, Chisti Y, Banerjee UC. Synthesis of metallic nanoparticles using plant extracts. *Biotechnol Adv*, 2013; 31(2): 346–356.
20. Bar H, Bhui DK, Sahoo GP, et al. Green synthesis of silver nanoparticles using *Jatropha curcas* latex. *Colloids Surf A Physicochem Eng Asp*, 2009; 339(1–3): 134–139.
21. Marambio-Jones C, Hoek EM. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *J Nanopart Res*, 2010; 12: 1531–1551.
22. Vigneshwaran N, Kathe AA, Varadarajan PV, Nachane RP, Balasubramanya RH. Functional finishing of cotton fabrics using silver nanoparticles. *J Nanosci Nanotechnol*, 2007; 7(6): 1893–1897.
23. Arora S, Jain J, Rajwade JM, Paknikar KM. Interactions of silver nanoparticles with primary mouse fibroblasts and liver cells. *Toxicol Appl Pharmacol*, 2009; 236(3): 310–318.
24. Prabhu S, Poulose EK. Silver nanoparticles: Mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *Int Nano Lett*, 2012; 2(1): 32.
25. Gurunathan S, Han JW, Dayem AA, Eppakayala V, Kim JH. Oxidative stress-mediated antibacterial activity of graphene oxide and reduced graphene oxide in *Pseudomonas aeruginosa*. *Int J Nanomedicine*, 2012; 7: 5901–5914.
26. Lara HH, Garza-Treviño EN, Ixtepan-Turrent L, Singh DK. Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. *J Nanobiotechnology*, 2011; 9: 30.
27. Chen X, Schluesener HJ. Nanosilver: A nanoproduct in medical application. *Toxicol Lett*, 2008; 176(1): 1–12.
28. Franci G, Falanga A, Galdiero S, et al. Silver nanoparticles as potential antibacterial agents. *Molecules*, 2015; 20(5): 8856–8874.
29. Chaloupka K, Malam Y, Seifalian AM. Nanosilver as a new generation of nanoproduct in biomedical applications. *Trends Biotechnol*, 2010; 28(11): 580–588.
30. Ahamed M, Alsalhi MS, Siddiqui MKJ. Silver nanoparticle applications and human health. *Clin Chim Acta*, 2010; 411(23–24): 1841–1848.
31. Johnston HJ, Hutchison G, Christensen FM, Peters S, Hankin S, Stone V. A review of the in vivo and in vitro toxicity of silver and gold particulates: Particle attributes and biological mechanisms responsible for the observed toxicity. *Crit Rev Toxicol*, 2010; 40(4): 328–346.
32. Ivask A, Kurvet I, Kasemets K, et al. Size-dependent toxicity of silver nanoparticles to bacteria, yeast, algae, crustaceans, and mammalian cells in vitro. *PLoS One*, 2014; 9(7): e102108.
33. McShan D, Ray PC, Yu H. Molecular toxicity mechanism of nanosilver. *J Food Drug Anal*, 2014; 22(1): 116–127.
34. Durán N, Durán M, Jesus MB, Seabra AB, Fávaro WJ, Nakazato G. Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. *Nanomedicine (Lond)*, 2016; 12(3): 789–799.
35. Suresh AK, Pelletier DA, Doktycz MJ. Relating nanomaterial properties and microbial toxicity. *Nanoscale*, 2013; 5(2): 463–474.
36. Fadeel B, Farcal L, Hardy B, et al. Advanced tools for the safety assessment of nanomaterials. *Nat Nanotechnol*, 2018; 13(7): 537–543.
37. Park EJ, Yi J, Kim Y, et al. Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism. *Toxicol In Vitro*, 2010; 24(3): 872–8.
38. Hadrup N, Lam HR. Oral toxicity of silver ions, silver nanoparticles and colloidal silver – A review. *Regul Toxicol Pharmacol*, 2014; 68(1): 1–7.
39. Singh R, Shedbalkar UU, Wadhwani SA, Chopade BA. Bacteriagenic silver nanoparticles: Synthesis, mechanism, and applications. *Appl Microbiol Biotechnol*, 2015; 99(11): 4579–93.