

Synthesis Routes of Silver Nanoparticles and Their Influence on Size, Shape, and Biological Activity

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ABSTRACT

AgNP formulations are frequently assessed in the same nominal concentration of silver, despite differences arising in synthesis that can alter numerous characteristics that govern the exposure of a biological target. This important narrative review provides an evaluation of how these changes, taken together, impact biological activity. Scope comparison of the seven previous reviews is undertaken, and another PubMed search is documented where all 388 unique records obtained from four focused searches are screened. The selected primary research is analyzed in terms of controls and measures used for exposures in order to justify the conclusions drawn by the authors. Two major issues, which arise repeatedly, are described in numbers: changing of potency ranking with the change of the dose metric and difference between administration concentration and actual dose. There are also changes in the properties of surface coatings when they come into contact with biological solutions, and formation of a corona can make the difference between the primary particles undetectable. Extracellular ionic regulation does not help solve the problem of silver release inside cells. In studies on antimicrobials and cancer research, activity should be taken into account together with cytotoxicity toward the host system; data on clinical wounds pertain to full preparations. This review highlights those comparisons that may differentiate a synthesis effect from changes in the means of delivery or residual components or interference in the assays. Reproducible synthesis, host compatibility, and environmental efficacy continue to be requirements in optimizing the applications.

Keywords: *silver nanoparticles, controlled synthesis, surface chemistry, delivered dose, protein corona, selective toxicity, critical review*

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INTRODUCTION

Biological comparison of two AgNP formulations starts from the materials science question: what is different about these two formulations? AgNPs are investigated for their application in antimicrobial therapy, wound dressings, and selective toxicity. Core size and composition of the particle surface are the parameters that can be altered to change the activity of AgNPs [1, 2]. However, a formulation that exhibits a higher activity in a microorganism or cancer cell test may also harm host cells. Thus, the relevant comparison should define the reasons for such activity and its implications for host-tissue compatibility. The synthesis method can provide significant control over the initial particles. The processes of seeded growth and polyol reaction create certain sizes or faceting, whereas laser ablation and biological assistance provide other surface

environments [3, 4, 5, 6]. Continuous flow and inverse design deal with the problems of mixing, residence time, and storage [7, 8]. These control mechanisms are not mutually exclusive. A change intended to reduce core size can also alter ligand coverage, residual reagents or colloidal stability. Purification adds another source of variation by changing the constituents retained in the tested dispersion. Attributing a biological result to diameter alone consequently requires evidence that these accompanying differences have been accounted for.

Characterisation of the prepared dispersion does not fully describe the material during an assay. Agglomeration, corona formation, dissolution and chemical transformation can change both its access to cells and the forms of silver present [9, 10]. cellular differences

associated with the chirality of the original coating disappeared after protein-corona formation [11]. For such a comparison, a coating measurement in water cannot establish which interface governed the cellular response. Measurements in the exposure medium are needed to connect the prepared surface with the observed effect.

The unit used to express exposure can change the interpretation even when the particles themselves are unchanged. At a fixed mass of silver, particle number and geometric surface area vary with diameter. The relative cellular doses associated with different sizes depend on the chosen metric, and a corresponding change in antibacterial potency rankings occurs after dose normalisation [12, 13]. Transportation and dissolution provide yet another criterion to differentiate between the addition of concentration in a system and the particulate/ionic silver that enters the cells [14]. The nominal concentration alone is thus an inadequate parameter for evaluating the inherent biological effectiveness of different formulations. Also, the measured endpoint should support the claimed effect. There might be nanoparticle interference in optical or metabolic assay signals, and this issue should be sorted out first in order to interpret any change as cellular damage [15]. For tumour models, the cytotoxic selectivity is contingent upon the reaction of an appropriate nonmalignant comparator under similar conditions of exposure [16]. The wound study addresses a different question, since it tests an actual product along with wound care. Any alteration in the dressing matrix or other ingredient prevents the clinical outcome from revealing the mechanism of synthesis.

The reported results did not convincingly confirm an enhancement of complete healing; the main endpoint and its margin of uncertainty have to be kept in mind [17, 18]. For a preparation, good performance needs to last more than the initial test. Optimization of the process to an outcome and variability in batches form a reproducibility issue for the biological

formulation as well [19]. The repeated exposure issue is another one where bacteria may adapt their response to nanosilver preparations over time [20]. Similarly, comparisons with the environment depend on how it is measured. Yield, energy cost and purification might outweigh the benefit gained simply from the source of the reductant [21, 22]. The application has to be known first for increased activity to be an improvement.

The links between preparation, exposure and response are already recognised in the review literature. Dissolution, ageing and incomplete exposure characterisation cover synthesis, medical applications and biosafety [1, 9]. Biosynthesis, stability, toxicity and synthesis–property relationships receive complementary treatment in [2, 23, 24]. [25] Connect preparation with corona formation, ion release and biological action. Address plant-mediated wound formulations through a process–property–performance framework that includes matched controls, silver speciation, dose reporting and study-design limitations [26]. The causal perspective used here is therefore established in earlier reviews.

Our contribution is to examine what those principles permit a reader to conclude from individual comparisons. We follow a deliberate synthesis change through material characterisation and exposure behaviour to the biological endpoint. Dosimetry studies of worked examples illustrate the occurrence of a seemingly apparent difference that can be explained by the dose definition, while other primary studies provide other explanations that either get answered or left open for consideration by the existing controls. The scope encompasses metal AgNPs and their conversions. The inclusion of salts, composites, and hybrid clinical formulations occurs where helpful in mechanism interpretation or translation. Figure 1 presents the sequence of questions used throughout the review. Table 1 compares this application with seven representative reviews and makes the overlap with earlier work explicit.

Table 1. Position of the present synthesis relative to selected earlier reviews.

Earlier review and focus	Relevant coverage	Operation undertaken in the present review
Transformation and toxicity [9]	Dissolution, ageing, coatings, biomolecular interactions, dose interpretation and experimental-design recommendations.	Apply these established principles to selected later controlled comparisons and connect each causal claim to a rival explanation, required experiment and remaining inferential limit.
Medical applications and biosafety [1]	Synthesis routes, antimicrobial and anticancer mechanisms, wound repair and cellular/organ toxicity; size, dose and exposure-time effects.	Separate activity from therapeutic selectivity by requiring matched host comparators and exposure conditions; distinguish mechanistic cell studies from evidence supporting a clinical formulation.
Biosynthesis and biological applications [2]	Source organisms, preparation conditions and characterization alongside antimicrobial activity, cytotoxicity and safety.	Evaluate extract or polymer carryover and purification as alternative explanations; require formulation controls before attributing activity or safety to a biosynthetic particle.
Interacting nanosilver actions [25]	Connects synthesis, characterization, corona and ion release with application-specific biological effects; proposes an action-network framework encompassing particle number, spatial range and interparticle interactions.	Apply the established framework to recent comparisons, quantitative delivered-dose examples and a common matrix of competing explanations, required controls and bounded conclusions.
Synthesis, structure and properties [23]	Route-specific methods and trade-offs, morphology and structure, physical properties and antimicrobial applications.	Distinguish the property deliberately changed during synthesis from properties that change with it, and evaluate whether exposure measurements support a size-, shape- or route-specific biological claim.
Synthesis variables, applications and toxicity [24]	Synthesis factors, capping, stability, dissolution-related parameters and human/environmental toxicity.	Join these factors to delivered dose and assay controls, then separate mechanistic support, in vitro selectivity and formulation-specific clinical evidence without a pooled efficacy ranking.
Plant-mediated wound formulations [26]	Process-property-performance framework; synthesis reporting, release/speciation, exposure, matched controls, phytochemical-corona attribution, cytocompatibility and study-design limitations.	Apply established causal and control principles across synthesis routes and biological models, adding quantitative delivered-dose examples and a competing-explanation matrix for selected primary comparisons.

The comparison is between seven purposively selected reviews and the analytical operation employed here. This is not a comprehensive coverage audit, nor a quality ranking, nor a claim that certain concepts or controls did not exist in previous literature.

Selection and Evaluation of Evidence **Search scope and update documented**

This critical narrative review was conducted through identifying relevant evidence to answer a series of specific questions regarding the

preparation, characterization, dissolution, dosimetry, bioactivity, and safety of AgNPs. Evidence was first identified in targeted PubMed and journal searches for foundational studies and methodological guidance and then

via back searching of references. This body of evidence was not systematically derived and is therefore purposeful.

A further PubMed E-utilities search was conducted on October 14, 2026. Four modules covered previous reviews (R), cellular dosimetry (D), dissolution and cytotoxicity in relation to corona or sulfidation (T), and clinical evidence from wounds (C). The period for R and T was between 1 January 2020 and 14 October 2026. The period for D and C was from 1900 to 14 October 2026 to include earlier studies. All records returned by each query were retrieved, i.e. no relevance-rank truncation was applied. The 389 record instances were deduplicated to 388 unique PMIDs. All titles and the 387 available abstracts were screened; the remaining record was a corrigendum without an abstract. Appendix A includes exact queries, API settings, counts and record level dispositions.

Eligibility selection and extraction

Articles that related AgNP production or the identity of the material itself to exposure, response, or translation were eligible for inclusion as review articles. As far as original studies go, an explicit definition of preparation, transformation, or dose, which was important for a biological effect or bioavailability or measuring it, was needed. Process and method articles relating to reproducibility or the validity of an assay or environment comparisons were also included. For the clinical section, only comparative studies of human wounds, burns, or skin outcomes using formulations the authors referred to as AgNPs or nanocrystalline silver were eligible.

Exclusions entailed unrelated material or endpoints, reviews in modules aimed at identifying the primary studies, protocols lacking results and clinical studies beyond the specified scope. Withdrawn or retracted articles did not qualify for use as evidence. In the case of relevant reports, inclusion into the analysis hinged on whether a given study had the capacity to disprove an alternative explanation or provided an alternative example; inclusion was not contingent upon the eventual citation. The articles retrieved contributed 18 citations. Another 274 relevant articles were not chosen for the bounded narrative. This does not take into account the core references as well as the

articles obtained via complementary citation searches but which were not contained in the four searches.

For each selected study, the following information was obtained for each study material, coating type, biological model, control group, dose unit, duration of exposure, and endpoint, along with any reported uncertainty. Numerical values from the report or abstract were confirmed by the available original publication or abstract, with standard errors kept as reported and not recalculated where confidence intervals were not reported. Bibliographic details were cross-checked using PubMed or the article publisher, with no language restriction applied to retrieval, although source text had to be in English. Where manuscript or proof copies of the paper were used for this appraisal, that is indicated.

Claim-Specific Limits and Appraisals

The assessment of the comparisons is made for six mechanistic/dosimetry comparisons and four clinical trials. In all cases, we analyzed what was being compared and the controls that make sense of it. We identified whether the comparison was based on measurement or modeling of exposure and any unresolved explanations. Access became an issue for this appraisal in the sense that an abstract that failed to describe the procedure was not able to prove the non-existence of the procedure.

Adequate retrieval of all records generated by the above queries is no guarantee of coverage for the topic. Several factors limit this study including the database and language selected, purposive sampling of the key references, as well as the accessibility and full report in English language. This study did not involve prospective registration and duplicate blind screening. We did not generate a pooled estimate for the effect size, and thus the clinical examples cannot estimate a combined treatment effect size. Variations in the formulation, media, dosage units, model, and end-points make any comparative potency ranking impossible. The graphs generated are geometrical representations of the assumed relations.

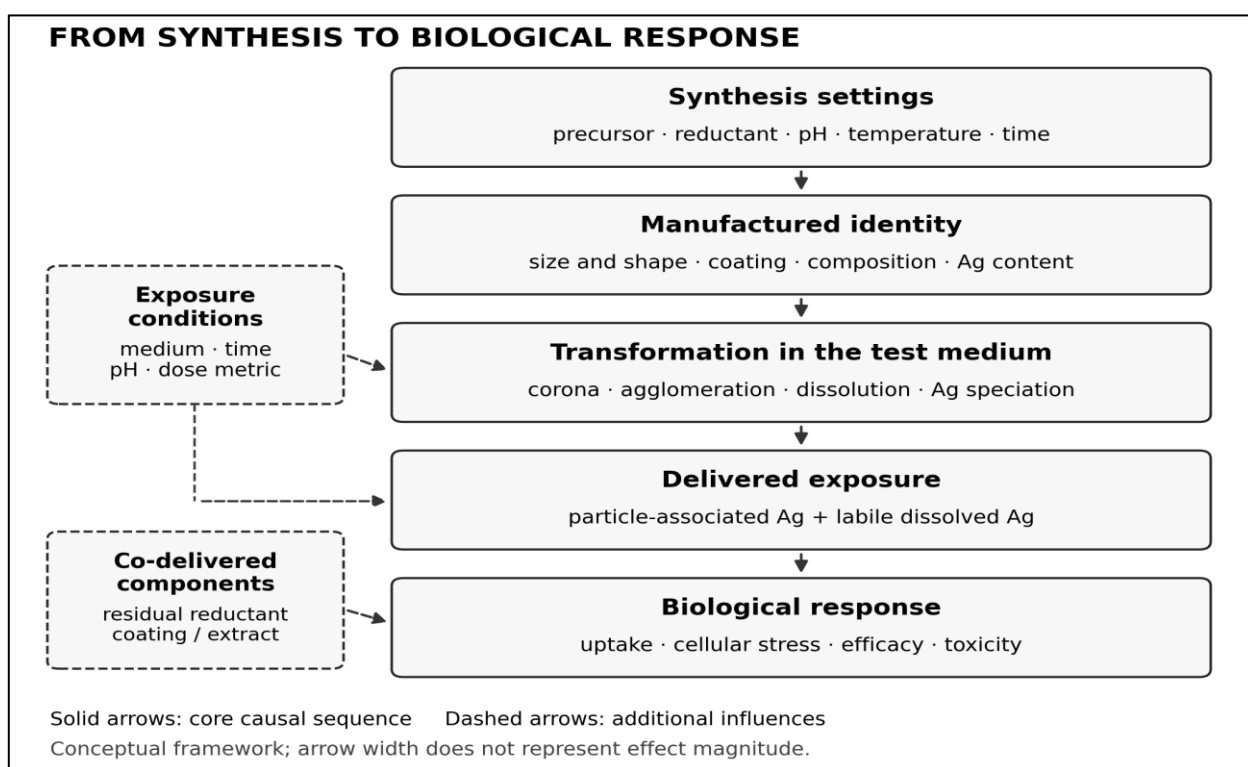


Figure 1. A Causal Framework for AgNP Synthesis and Biological Response. *The synthesis determines the primary material identity, while the test medium can affect the corona, agglomeration state, dissolution kinetics and silver speciation. The delivered exposure may comprise particle-associated silver and labile dissolved silver, operational categories that require appropriate measurement. Exposure conditions and co-delivered formulation components introduce additional pathways to the measured response. The scheme is conceptual, does not imply any one dominant mechanism universally, and does not assign effect sizes.*

Synthesis variables and manufactured particle identity

Reduction chemistry and controlled growth

Synthesis determines core populations, surface species, and co-delivered components. A controlled comparison must determine which of these properties is intentionally changed and which is changed incidentally to it.

Seeded synthesis is particularly useful in this respect. Spherical particles of 10–200 nm have been prepared by kinetically controlled growth and citrate stabilisation [3]. Families of related particles can decrease the number of uncontrolled differences in a size comparison. But analogous nominal chemistry does not ensure identical ligand coverage per unit surface area or identical dissolution. We must assess those properties before we can attribute the comparison specifically to diameter.

Temperature or alkaline conditioning of tannic acid could modify subsequent silver particle formation [27]. It is thus relevant to consider the history of the reductant preparation irrespective of whether the name remains the same. For a reproducible procedure, record not only the concentration and order of addition but also the pre-treatment, pH, and reaction temperature. This issue also concerns biological extracts, which might vary because of the process of extraction and storing prior to being added to the silver precursor.

Single-crystalline silver nanocubes have been made by Sun and Xia via polyol process in the presence of polyvinylpyrrolidone (PVP) where the shape of the product is influenced by the conditions of synthesis [4]. The controlled preparation allows for comparison of the shapes, but the comparison of cube to sphere would not be clear if the different shapes have been

prepared with a difference in surface coating, leftover reagents or behavior in the exposure medium. Pictures would show that the shapes are different. They would not show what caused the biological effect.

Radiation and electrode induced approaches

With respect to the photoinduced reaction analyzed, citrate concentration, irradiation time, and UV intensity were preparation variables [28]. It is not sufficient to only give information on the power of the light source, since the geometry of the illumination and the irradiation time also have to be reported. Optical spectra can be obtained after the reaction; however, microscopy should be done for structure identification.

The electrochemical reduction technique can help minimize the use of a chemical reductant while maintaining the other components of the formulation. PVP in sonication-enhanced electrochemical synthesis showed better dispersion and minimized electrode fouling [29]. When using biological studies as comparisons for different methods of synthesis, the coating should be properly matched, and electrolyte carryover should be established. A further way to get particles without deliberately adding an organic reducing agent is laser ablation in liquids. The liquid is still involved in the formation of the surface. Compared silver particles produced in water and in lithium chloride solution showed different antimicrobial behaviour [5]. So, solvent and electrolyte choice, as well as surface and dissolved-silver measurements, should be included in the comparison. Ligand-free means no ligands deliberately added, not a surface free of adsorbate during exposure.

A useful comparison of efficacy versus toxicity for laser-generated silver nanoparticles stabilised with citrate is provided by [30]. A different antimicrobial response was observed when tested preparations with mean transmission electron microscopy (TEM) diameters of about 21 and 15 nm were employed. The smaller-particle preparation was

active against several tested strains at 14.35 $\mu\text{g/mL}$, but also reduced dermal fibroblast viability at that concentration. Simultaneous fibroblast response limits the useful window of activity. Preparation characteristics co-vary, so the response differences are not attributable to diameter alone.

Flow processing and reproducible quality targets

Obtained similar particle sizes in continuous-flow and droplet reactors at low Dean numbers when hydrodynamic conditions were matched [7]. Moreover, the droplet process also needed oil separation. While flow processing may make mixing and residence time clearer compared to batch mixing that is poorly understood, this comparison highlights the importance of the operating regime in this case. There is a need for consideration of factors such as collection, fouling, recovery, and stability in the choice of a reactor.

Mixing can influence formation on a much shorter time scale than that shown by bulk sampling. An inkjet mixing system was used, where droplet collision conditions affected the size of silver particles [31]. This supports reporting the mixing operation itself rather than treating reagent concentrations as a complete specification for synthesis. A complementary approach to control is offered by inverse design: combined reaction, hydrodynamic and storage descriptors to predict preparation conditions associated with a target size [8]. Such predictions need to be validated in their chemical and operational domain and then tested independently in biology.

Quality targets should be application-specific. Optimised microwave synthesis based on surface-enhanced Raman scattering performance and variance, reporting intra- and inter-batch variability of less than 15% at the validated operating point [19]. The lesson that generalises is to define an endpoint and acceptable variability before optimising a process. Release behaviour, dispersion stability,

activity and host compatibility can augment or replace optical performance for biomedical use. Reproducibility cannot be proven by repeating the measurements of one batch; independent batches of synthesis are required.

Biological inputs and purification

Biological synthesis is a chemical transformation that is done with biologically derived reagents. Its reproducibility depends on the production and handling of those reagents. Illumination of cell-free yeast extract altered the yield and the particle size [6]. Hence, cultivation, extraction, and light exposure are controllable process inputs, not incidental details. Also required is an extract-only comparator in case the product dispersion contains antimicrobial or cytotoxic compounds. Defined biopolymers can help with certain aspects of controlling inputs, but they also have their own interfacial behaviour. silver formulations with hydroxyethyl cellulose and a

design of experiments approach [32]. Their work also helps to clarify the need to distinguish core and dispersion measurements. One formulation had a TEM core diameter of 16 ± 1 nm and a dynamic light scattering (DLS) diameter of 286 ± 4 nm. The latter is the hydrodynamic behaviour of the dispersed population, not a second measurement of the same dimension of the metallic core. Purification is part of synthesis for biological interpretation. Removal of unreacted precursor, free reductant or excess extract may influence both the measured activity and colloidal stability. Unpurified preparations can cause confusion between particle activity and soluble carryover effects. Hence, besides the yield of the synthetic compound, the quantity of silver recovered, the washing conditions, and the stability of the dispersed phase after purification need to be recorded. Table 2 highlights the route specific comparisons of the issues involved without declaring a winner

Table 2. The synthesis processes involved in making the comparison biologically valid.

Route	Process variables	Potential comparison problem	Evidence needed
Seeded reduction and polyol	Seed-to-precursor ratio, reductant conditioning and ligand ratio [3, 4, 27]	Changes in geometry, ligand coverage, and reactants can occur simultaneously.	Core distributions, surface structure, purification and release under identical exposure conditions.
Laser ablation in liquids	Liquid composition, pulse conditions, and stabilization after synthesis [5, 30]	The lack of added reductant does not mean an inert surface.	Release of silver, particle-ion comparisons, and effects of hosts at antimicrobial concentrations.
Flow and droplet processing	Mixing, residence time, and Collection and Storage [7, 8]	These processes may affect the quality of products.	Independent-batch variation, recovery yield and dispersion stability after collection.
Photo and electrochemical synthesis	Irradiation history, electrolyte, electrode condition and stabilizer [28, 29]	Energy input does not establish ligand-free composition or improved bioactivity.	Defined energy conditions, final surface composition and appropriate formulation controls.
Extract and biopolymer assisted synthesis	Extraction, illumination, ratio of polymers and purification [6, 32]	Input variability and residual soluble components confound particle effects.	Input characterization, extract or polymer controls, recovered-silver yield and direct safety tests.

The comparison is a synthesis of the cited primary evidence. No route is assigned a universal safety or performance ranking.

Particle identity in the exposure medium.**Core size, shape and the limits of optical characterization.**

TEM reports on the projected core dimensions and shape heterogeneity while DLS reports on the diffusion-related hydrodynamic behaviour which can be strongly influenced by larger scattering objects. The disagreement between them can reveal dispersion behaviour rather than justifying the choice of the value that best supports a size–activity claim. Dispersion characterisation also needs to take into account the exposure medium: measurements in water alone are not enough for biological experiments containing salts, proteins and a different pH [32, 33].

However, structural change can be monitored by localised surface plasmon resonance (LSPR). optically and structurally characterised the photoinduced conversion of silver nanospheres into nanoprisms [34]. However, a plasmon maximum is not a definitive size measurement: the spectrum can be altered by shape mixtures, local refractive index and interparticle interactions. UV–visible spectroscopy can therefore assist in the evaluation of synthesis and stability but does not determine purity, ligand identity or biological compatibility. Triangular particles were more antibacterial than the alternatives tested [35] and spherical particles were most effective in inhibition-zone experiments [36]. These results need not be in conflict, as the formulation and assay conditions were different and inhibition zones are a reflection of transport through agar as well as activity. They do not define a universal order of shapes. [13] demonstrated explicitly a dependence on dose metric, where the relative antibacterial potency of cubes, discs and pseudospheres changed when the exposure was expressed as mass, particle number or surface area. Conversely, [37] reported different antibacterial effects without a significant shape dependence of cytotoxicity in their human mesenchymal stem-cell model. Therefore, a

shape-activity statement must identify the biological target, exposure metric, and endpoint.

Dissolution and silver speciation.

Liu and Hurt studied the dissolution of silver from model aqueous colloids consisting of metallic, adsorbed and dissolved silver in which dissolution was a function of the presence of oxygen and protons [38]. Later, [39] changed dissolution behavior by modifying the surface. It is thus apparent that both the particle surface and the receiving medium play roles in the process of dissolution. A dissolution rate determined for one particular medium cannot be applied to another due to ligand, protein competition and other factors.

The silver fraction resulting from the elemental analysis procedure is dependent upon the process used for removing the particles. The effect of losses during the process, silver that is captured by the separating mechanism, and very small particles that remain in the sample are all potential factors that will impact the result. It is important to report the separation procedure used and the recovery rate along with the chemistry of the medium to understand this operational fraction because it does not indicate available or labile silver. In the anaerobic state only, [40] prevented the oxidation release and recorded minimal particle-specific toxicity in the *Escherichia coli* system used. This indicates an ion-based mode of bacterial inhibition in these conditions. Additional contributions are included in other models: [41] implicated surface defects and direct contact in plate-associated hazard in fish gill cells and zebrafish embryos; and responses to particles and extracellular silver cations were differentiated in mammary cell models by [42]. The relative contributions of particle uptake, localised transformation and ionic exposure thus remain system dependent.

Intracellular dissolution of particles cannot be excluded by a negative extracellular-ion comparison. Particles can modify the time and place of silver delivery; equal amounts of silver added as salt and particles do not necessarily

translate to equal intracellular exposure. However, agglomeration was substantial, and the increased cytotoxicity of 10 nm particles was ascribed to increased silver release rather than a clear size-dependent uptake difference [43]. Thus, particle and ionic mechanisms should be regarded as potentially coupled exposure routes.

Ionic controls also need matched preparation histories. demonstrated that silver acetate aged for at least three hours in serum-containing culture medium caused little 24-hour lactate dehydrogenase (LDH) injury in RAW 264.7 macrophages, while fresh controls and particle-dissolution supernatants were toxic [44]. Therefore, a preaged bolus may not mimic sustained ion release from particles. The model's cellular ion dose was more consistent with response than particle metrics. Elemental analysis measured total silver and the model assumed extracellular origins for cellular ions, not directly resolving intracellular dissolution. This is consistent with time-matched controls, not universal ionic safety, and not definitive exclusion of an intracellular pathway.

The surface layer is dynamic.

The first coating may be different from the interface encountered by the cells after the association of proteins with the surface, changing agglomeration or participating in transformation. [10] showed that dynamic protein coronas modulated the sulfidation of silver nanoparticles in vitro. The corona was a reactive environment, which supports characterisation after relevant incubation periods and at the actual particle to protein ratio. [45] studied 40 nm silver cores coated with citrate, branched polyethyleneimine or polyethylene glycol and observed coating-dependent albumin interactions and HepG2 responses.

[46] found increased toxicity in HepG2 cells after citrate-coated particles were exposed to native or glycated albumin, although a reduced dissolution was measured separately in a defined buffer. The different media prevent this observation from excluding intracellular silver release and show the need to evaluate

dissolution and cellular response in similar environments. Associated uptake and cytotoxicity with surface charge in their formulation library, while also finding that the corona affected dispersion behaviour [47]. This result identifies charge as an important variable within that library; it does not establish its priority over protein identity in other formulations. For capping-agent comparisons, the relevant surface is the one present during exposure, which may differ from the surface whose nominal charge was measured in water.

Another test of the endurance of constructed identity under exposure is surface chirality. [11] compared pristine AgNPs with those coated with L-cysteine, D-cysteine, and racemate. L-cysteine-coated particles showed higher uptake and cytotoxicity in J774A.1 cells than the D-coated formulation, but the differences reported were removed by protein-corona coverage. The authors described similar patterns of organ distribution following intravenous administration in mice. Thus the abstract supports corona-dependent masking of the cellular differences tested; it does not prove equivalent organ toxicity, long-term safety or formal equivalence between formulations. Dose, sampling times and uncertainty were not assessed from the abstract. Extract-derived preparations may contain tightly associated constituents, loosely bound species and soluble residual extract, each with differing access to the target. An infrared band can identify a chemical group in the sample, but it does not quantify the amount of a specific molecule that is surface bound. Additional descriptors related to manufactured identity and identity during exposure are provided in Table 3.

Table 3. Complementary measurements connecting manufactured identity to exposure identity.

Descriptor	Useful measurement	What it supports	Limit on inference
Core geometry	TEM or suitable electron microscopy	Core dimensions, morphology and population heterogeneity	A dried image does not establish the hydrodynamic state during exposure [33].
Dispersion state	DLS with distribution and medium details; complementary imaging	Hydrodynamic behavior and changes after mixing	Hydrodynamic diameter is not metallic-core diameter; large objects can dominate scattering [32].
Optical response	UV-visible extinction with concentration and path length	Changes in plasmonic response during formation or incubation	A peak is not a unique size, purity or safety measurement [34].
Surface and corona	Appropriate surface analysis and adsorbed-protein measurements	Evidence for the initial and acquired interface	Bulk functional-group signals do not uniquely quantify bound ligands [45, 46].
Total silver	Validated elemental analysis after digestion	Silver concentration and recovery	Elemental mass alone does not identify particles or chemical species [12].
Released silver	Time-resolved separation with recovery checks and elemental/speciation analysis	Operational dissolved fraction and changes with medium	Dissolved total silver is not automatically free Ag ⁺ ; separation can alter the sample [14, 38].
Cell-associated dose	Elemental mass plus localization or complementary uptake measurements	Material reaching or associated with cells	Association and deposition do not by themselves establish internalization [12].
Blood and medium compatibility	Relevant whole-matrix assays with interference controls	Response of the intended biological environment	Water stability or a natural coating cannot substitute for matrix-specific testing [48].

Measurements should refer to the actual concentration, medium and incubation interval whenever practicable. This is an author-synthesized comparison, not a certification standard.

Dose comparability and reliable biological measurement.

Equal mass is not equal particle exposure.

For ideal monodisperse, nonporous spheres with diameter d , silver density ρ and total silver mass m , the particle number N and total geometric surface area A are given by the following identities:

$$N = 6m / (\pi \rho d^3) \quad \text{and} \quad A = 6m / (\rho d) \quad (1)$$

For equal mass and density, comparison with a reference diameter d_0 gives:

$$N / N_0 = (d_0 / d)^3 \quad \text{and} \quad A / A_0 = d_0 / d \quad (2)$$

For a constant amount of silver mass, reducing the diameter from 20 to 10 nm increases the number of nanoparticles by a factor of eight and doubles the geometric surface area. These two identities are illustrated as functions of size from 10 to 100 nm in Figure 2. No biological end-point was fit or extrapolated for either identity. It is important to consider the assumptions behind the use of these two identities in a preparation.

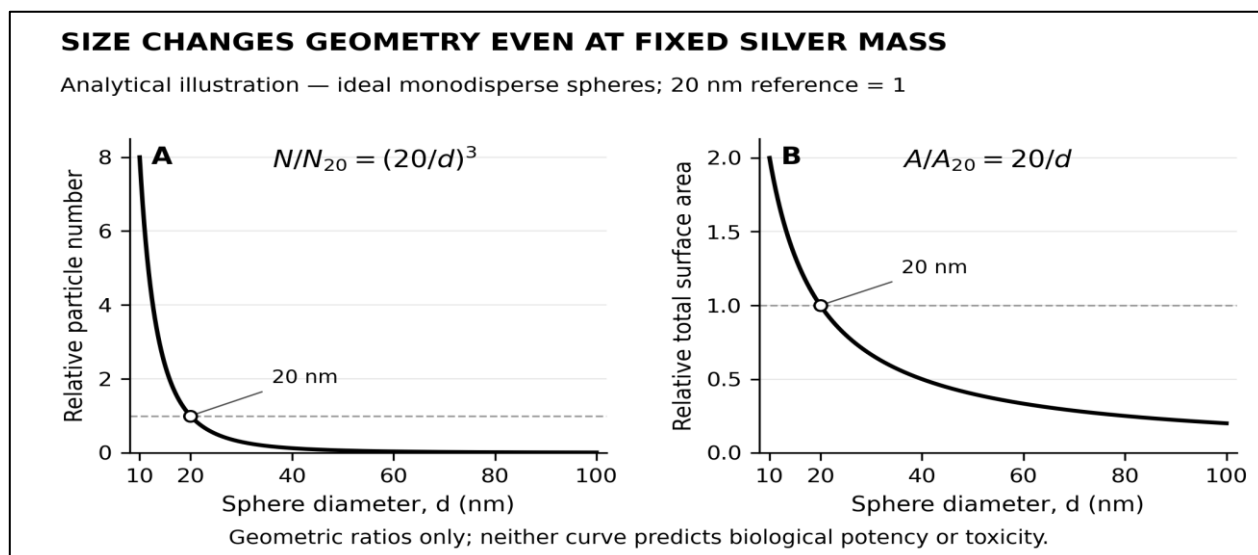


Figure 2. Geometric scaling for the ideal case of monodisperse spherical Ag particles having a constant total mass and density of Ag. (A) Number of particles as a function of the reference 20 nm diameter, $N/N_{20} = (20/d)^3$. (B) Total surface area of particles as a function of reference 20 nm diameter, $A/A_{20} = 20/d$. The open circles represent the reference diameter. The scaling holds for isolated, non-porous spheres having the same density of Ag and does not take into account coating mass, anisotropy, agglomeration, dissolution or uptake by biological systems.

The differing potency rankings demonstrate the effects of the selection of a dose basis [13]. If the masses of the silver are held equal, then comparing diameters would imply a different number and surface area. Comparing the same on a fixed number and/or fixed surface area basis is a different problem and might involve the use of different masses of silver. The same applies to the IC₅₀, which is the concentration that yields a 50% decrease in the given assay response.

Administered, deposited and internalized dose.

Sedimentation, diffusion and dissolution modify the administered particle population and its delivery. The effective agglomerate density is important for transport and cannot always be replaced by bulk silver density [49]. Because material arriving at a cell layer may remain extracellular, administered, deposited, cell-associated and internalised silver require distinct terminology and measurements. [12] studied 20 and 75 nm citrate-coated particles in ARPE-19 cells using flow-cytometry side scatter, deposition models and inductively coupled plasma mass spectrometry (ICP-MS) of

separated fractions. Both models overestimated deposition, although their estimates were closer to total deposited silver than to silver in the isolated cell fraction. Particle numbers and surface areas were calculated from elemental mass under a uniform-sphere assumption that ignored agglomeration. The resulting ranking concerns those dose estimates, rather than toxicity. Each dose was denoted by a single flask, while the analytical replicate ICP-MS analysis was technical rather than biologically independent. Neither the sum of elemental silver nor the deposition model is able to distinguish intracellular particulate silver by itself.

Calibrated the In Vitro Sedimentation, Diffusion, Dissolution, and Dosimetry (ISD3) model using time-dependent silver concentrations from 20 nm and 110 nm preparations [14]. The model couples particle transport, silver release and ion delivery, so its exposure estimates depend on parameters for each process. Changing the coating or medium requires those parameters to be supported and the predictions validated under the new conditions. Agreement with terminal viability

alone would leave the predicted particle and ion doses untested.

[50] validated the ISD3 against static-cuvette sedimentation and provided a quantitative worked example. The modelled 24-hour target dose was around 85% lower than the nominal concentration for their 96-well human umbilical vein endothelial cell (HUVEC) and HepG2-derived C3A cultures. When the same viability data were plotted against the modelled target dose, the estimated IC₅₀ changed from 41.04 to 8.13 µg/mL for HUVEC and from 54.78 to 10.95 µg/mL for C3A cells. This is not an improvement in terms of dose rate but rather a change in dose basis. This model presumes instantaneous uptake at the cell surface and does not account for intracellular dissolution; thus, it cannot be related to actual uptake.

Interference and control logic.

Particle absorption, scattering, adsorption of reagents on the particles, and reaction between particles and reagents may all add to the optical signal besides the cell concentration. Cell-free particle/reagent controls reveal the contribution of those factors in the assay; independent biological control determines if there is any validity of the cellular response that one has deduced. Corrections need verification over the range of concentrations, coatings, and time periods that one has exposed the cells to the reagents. Showed formulation-dependent

effects on the MTT, Neutral Red, Hoechst, and resazurin assays [15].

The validation of an assay then depends on the specific material-reagent pair; one finding is not enough to determine whether the test is valid or invalid for all formulations of AgNPs. An increase in cell viability from the optical assay needs confirmation through another biological assay.

A parent nanoparticle suspension could be compared to an ionic control, the corresponding coating or extract and a depleted fraction that is obtained using a validated procedure for separating the particles. Each comparison corresponds to the source of potential biological activity. An extract alone serves as a test for the possible role of co-formulation, but it is possible that it will show a different behavior if it is adsorbed on the surface; a soluble salt serves as a test for ionic activity, but it cannot replicate intracellular release.

Microbial contamination can be problematic in relation to inflammatory endpoints. In an inter-laboratory evaluation involving several nanomaterials, [51] found significant variability in endotoxin measurement, even with the distinction made although meeting the quality criteria of the test. While this was not specifically related to an AgNP effectiveness study, it does help validate endotoxin measurements through interference testing.

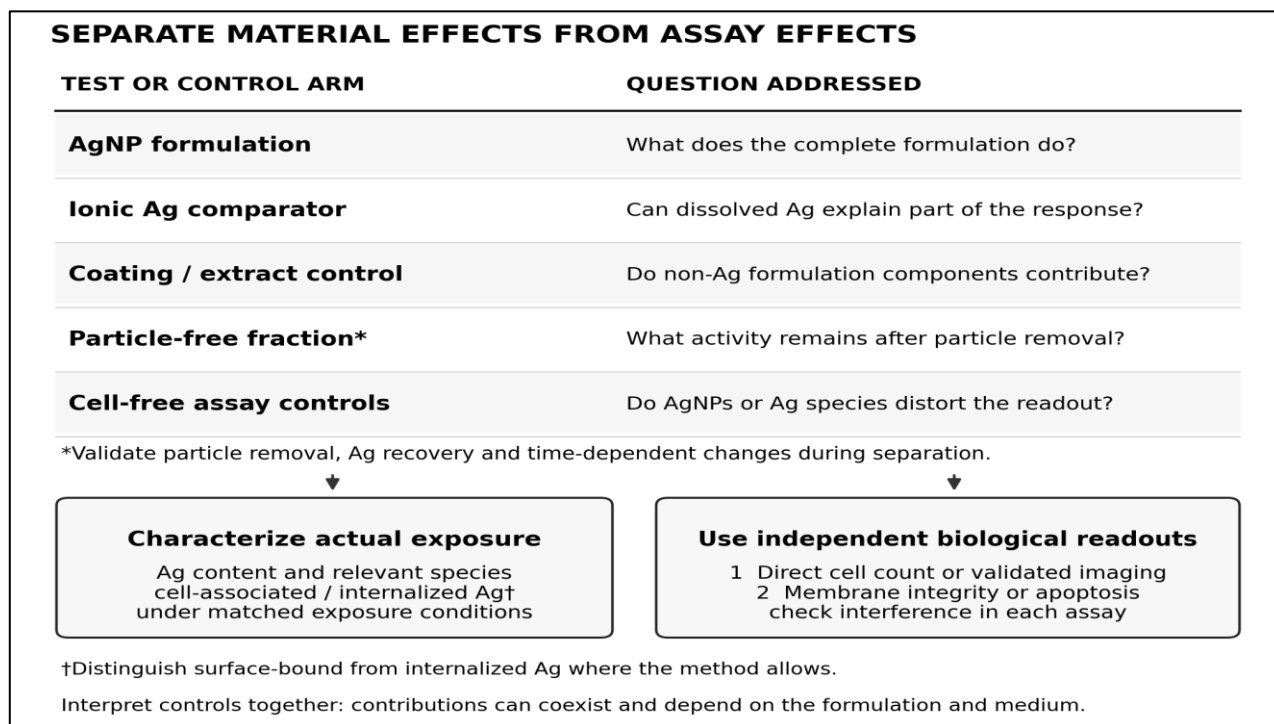


Figure 3. A control framework for separating particle-related, dissolved-silver, formulation and measurement contributions. The ionic comparator should be interpreted using relevant silver exposure and medium chemistry rather than nominal total silver alone. A particle-free fraction is informative only when particle removal, silver recovery and processing-related changes have been checked. Cell-free controls test optical and chemical assay interference. Exposure characterization and complementary biological endpoints should be interpreted together; no single comparator establishes an exclusively particle-mediated or ion-mediated mechanism. The biological readouts shown are examples for cellular systems and require application-specific validation.

Selectivity and reproducibility.

An IC₅₀ against a cancer cell line is a measurement of effect in a defined assay. It is not a measurement of a therapeutic window. A selectivity index may be defined as the IC₅₀ in a relevant nonmalignant comparator over the IC₅₀ in the tumour model, provided that concentration basis, exposure duration and endpoint are comparable. Its uncertainty depends on both fitted values, and a normal-cell IC₅₀ outside the measured range should result in a bound, rather than an invented point estimate. Otherwise differences in cell media, growth rates and exposure can lead to apparent selectivity. [52] proposed the Minimum Information Reporting in Bio–Nano Experimental Literature (MIRIBEL) guidelines as a basis for describing material, biological and

protocol variables. Applied here, the guidelines support reporting the synthesis batch as an independent unit, distinguishing technical replicates from biological replication, and stating how the particle distributions and concentration-response curves were analysed. Claim-specific comparisons in Table 4 reflect these considerations. The table is an analytical framework for this review, not a validated quality score or a substitute for application-specific experimental design.

Table 4. Claims and the comparisons needed to distinguish competing explanations.

Proposed claim	Comparison or control	Interpretable result	Remaining boundary
A size or shape effect	Related material family; mass and additional dose metrics; release measurements	A response difference evaluated against geometry and exposure [13]	Cannot isolate geometry if coating or medium behavior also differs.
A particle-associated mechanism	Ionic comparator, validated particle-depleted fraction, surface and exposure measurements	Evidence distinguishing uniform ionic exposure from particle-associated delivery [40, 42]	A salt control cannot exclude local intracellular dissolution.
A genuine cytotoxic response	Cell-free material–reagent controls and a complementary endpoint	Biological change remaining after assay interference is assessed [15]	An optical correction must be checked for each formulation and dose.
ROS-dependent cell death	Temporal measurements and perturbations with colloid checks	Rescue evaluated alongside changes in silver availability [53]	Thiol protection may change the material as well as cellular redox state.
Selective tumor toxicity	Relevant nonmalignant comparator with matched time, dose basis and endpoint	A comparative window with uncertainty or valid bounds [16, 54]	In vitro selectivity is not a clinical therapeutic index.
Resistance is suppressed	Repeated exposure, survivor testing and colloidal tracking	Stability of susceptibility under the tested regimen [20, 55]	A short adaptation experiment does not establish clinical resistance prevalence.
Manufacturing is reproducible	Independent synthesis batches and prespecified quality attributes	Batch consistency against an application-defined target [19, 52]	Technical replicate wells cannot substitute for independent batches.

ROS, reactive oxygen species. These comparisons are proposed inference checks. They do not constitute a formally validated risk-of-bias score.

Table 5 applies these inference checks to six primary mechanistic and dosimetry examples. It distinguishes directly measured exposure from model-dependent quantities and separates evidence for a response from proof of its mechanism.

Table 5. Claim-specific appraisal of mechanistic and dosimetry comparisons.

Study and tested contrast	Evidence supporting interpretation	Limit not solved; source accessibility	Conclusion
E. coli exposed to AgNPs under controlled oxygen conditions [40].	Strictly anaerobic synthesis/exposure suppressed oxidation; PEG- and PVP-coated size series were compared with AgNO ₃ dose-response.	A: the full set of control parameters and statistical methods was not re-evaluated. The organism, coatings, and exposure regime delineate the test range.	Silver release accounts for the observed antibacterial activity in this setup, but the experiment does not rule out other systems' particle-based effects.
Nominal 10–75 nm formulations in BEAS-2B lung cells [43].	Medium characterization, release and uptake measurements complemented two cytotoxicity assays and explicit assay-interference checks.	F: There is variation between coating and agglomeration on the panel. The inhibition of LDH will mask damage, and medium release cannot measure ionic silver intracellularly.	Increased toxicity at 10 nm is expected from dissolution effects, but there is no known size threshold or intracellular process that exists independently.
20/75 nm citrate AgNPs; ARPE-19 cells, 24 h. [12].	ICP-MS fractions, flow-cytometry scatter and deposition models; calculated number/surface-area doses.	F: one flask per dose; six ICP-MS measurements are technical replicates. The numbers/surface area calculation does not consider agglomeration; the total elemental silver does not have intracellular speciation.	They both overestimated deposition; cell dose, deposition mass, and assumed number of particles are three different things.
Fresh/aged silver acetate and citrate AgNPs grown on gold cores; macrophages [44].	Ion-aging series, dissolution supernatants, uptake-deficient cells, total-silver measurements and calibrated dosimetry modeling.	F: direct measurement of cellular ion species was not performed; the model considers extracellular ions and lacks intracellular dissolution explicitly.	Ionic control history alters the comparison. Good model fit allows dose hypothesis without establishing universal toxic species or ruling out intracellular release.
Nominal versus modeled target dose in HUVEC and C3A cultures[50].	Static-cuvette validation of transport predictions; viability curves expressed on two dose bases.	F: The process of cellular uptake continues to be modeled; instantaneous surface uptake is taken for granted while intracellular dissolution remains unexplained.	A change in dose basis will only affect the numerical IC ₅₀ value of the response; it will not enhance the compound or indicate its intrinsic potency.
PVP-coated AgNPs in paired pNF and Schwann cells[54].	Patient-matched lines, AgNO ₃ comparison and MTT, ATP and dye-exclusion endpoints support a differential response beyond one metabolic assay.	F: Both of these lines come from the same donor; further experiments will not provide an additional donor. The lesion created is harmless.	This model demonstrates selectivity for the tested formulation in vitro; selectivity in the population at large and the therapeutic window have yet to be determined.

F, relevant full-text sections examined; *A*, original abstract used for this appraisal. An access or appraisal limit is not evidence that the original study omitted a procedure. AgNPs, silver nanoparticles; PVP, polyvinylpyrrolidone; PEG, polyethylene glycol; LDH, lactate dehydrogenase; pNF, plexiform neurofibroma. These qualitative judgments concern specific claims and are not formal risk-of-bias scores. ICP-MS, inductively coupled plasma mass spectrometry; HUVEC, human umbilical vein endothelial cells.

Biological activity interpreted against the evidence.

Antimicrobial action and resistance.

MIC (minimum inhibitory concentration), MBC (minimum bactericidal concentration), inhibition-zone diameter, viable counts and biofilm biomass are measures of different processes and cannot be combined as interchangeable indicators of potency. Synthesis comparisons should detail the media, inocula, duration, basis for silver concentration and process controls. For comparisons using agar, diffusion and release also need to be considered when interpreting the results [35, 36].

[56] reported that small silver nanoparticles potentiated aminoglycosides. Nematode lifespan was not affected, whereas motility was affected, demonstrating the need for complementary endpoints in safety assessment. Before defining enhanced activity as synergy, combinations should be compared to single agents at matched concentrations using an explicit interaction model. Aggregation-related resistance in *E. coli* and *S. aureus* following exposure to sub-inhibitory concentrations, though extracellular resistance mechanisms were different for the two bacteria [55]. In the system investigated resistance developed from flagellin-induced precipitation to an efflux pump mechanism [20]. The above demonstrates how bacteria adapt during exposure to create a changed environment for the colloid to function. Thus, the stability of dispersion prior to exposure is only a point of departure for evaluating activity during repeated exposure. A different observation was made in a six-strain experiment in which three successive nanosilver exposures lead to an increase in the antibiotic susceptibility, and in addition, the susceptibility to nanosilver has increased in some strains [57]. The selection of the strain, treatment schedule, and outcomes are different in the current

experiment from the selection experiments for resistance, so the observations do not suggest any consistent response to repetitive exposures.

Tumor models and selective cytotoxicity.

[16] compared malignant and nonmalignant breast models, including three-dimensional and in vivo systems. The nonmalignant comparator permits assessment of differential sensitivity, which an isolated tumour-cell IC₅₀ cannot establish. These findings concern the tested formulations and preclinical models; they do not establish systemic safety or efficacy in patients. In mesenchymal triple-negative breast cancer models, [58] associated increased nanoparticle sensitivity with lipid peroxidation and proteotoxic stress. [42] distinguished particle-induced cell death from the response to silver cations in related mammary models. The first comparison concerns cellular subtype; the second concerns exposure form. Neither establishes selectivity across all tumours. A distinct response to particles also leaves open the possibility that particle-associated silver transforms after entering cells. Patient-matched controls are another useful design example. [54] compared paired immortalised plexiform neurofibroma and Schwann-cell lines from one patient with neurofibromatosis type 1 (NF1) and reported 72 h IC₅₀ values of 3.1 ± 1.0 and 31.2 ± 3.0 $\mu\text{g Ag/mL}$ for the tested PVP-coated formulation, respectively. The authors provided these uncertainties as standard errors. Plexiform neurofibroma is a benign tumour, and this evidence should not be incorrectly classified as a malignant cancer trial. The point estimates give a ~10-fold ratio, indicating an in vitro comparison and not a clinical therapeutic index. A separate 48-hour comparison with AgNO₃ failed to replicate the differential response; it differed in duration from the 72-hour nanoparticle IC₅₀ experiment. The result calls

for exposure-resolved testing rather than a direct therapeutic-window inference.

Mechanistic language should reflect what the experiments resolve. Oxidative injury, membrane damage, mitochondrial disturbance and regulated cell-death pathways can overlap, but an increase in fluorescence alone is not sufficient to establish their causal order. [43] investigated silver particle toxicity under conditions in which no increase in reactive oxygen species or nuclear localisation was found. In contrast, a rescue reagent can modify the chemistry of the particle. [53] found that thiol antioxidants interacted with the colloid and led to a different protection pattern than a non-thiol antioxidant. Thus protection by a thiol does not uniquely identify reactive oxygen species as the initiating mechanism. Measuring biological responses along with effects on dispersion and availability of silver makes the perturbation experiments more powerful.

What clinical wound studies do and do not establish.

Clinical wound trials test complete products with wound care and their results do not isolate an effect of silver core size or synthetic route. Other examples of different exposures are nanocrystalline dressings, mixed-metal dressings and sprays.

In the pilot study by [59], with 31 participants, 12-week healing was numerically higher with nanocrystalline-silver alginate than with Manuka honey or paraffin tulle, but the primary healing comparison was not significant and the confidence interval was wide [59]. In a larger

trial, [17] randomised 125 participants and analysed 118. Acticoat did not demonstrate increased 12-week ulcer healing compared to non-silver dressings plus standard care (69% vs. 75%; adjusted odds ratio 0.92, 95% CI 0.26–3.22). The findings do not demonstrate equivalence.

A pilot study of 30 patients comparing Ag/Cu with ionic silver. This study shows the difference between a promising trend and proven superiority. Findings at selected visits favored Ag/Cu, but there was no overall significant difference in wound-area decline ($p=0.151$); baseline areas differed substantially and the active phase was one week [60]. The copper and dressing architecture preclude an attribution to particulate silver alone.

74% against 62% Closure in 6 months without grafting using silver spray and negative-pressure wound therapy (NPWT) against control treatment; the difference noted was 12% (95% CI -6.1 to 30.1) [18]. The publicly available proofs by the author include saline control and blinded allocation but per protocol analysis after 15 losses. It also has discrepancies in the secondary estimates.

Table 6 summarizes how these four trials differ from each other in terms of product, outcome, and design issue. Positive responses in wound area alone cannot be used as a replacement for healing and safety. The results cannot be generalized beyond the products and wound conditions being studied here; this is not a full evidence review on the topic.

Table 6. Selected randomized wound trials: products, primary outcomes and limits of inference.

Trial and product comparison	Randomization and analysis	Patient outcome and uncertainty	Claim-specific appraisal
Acticoat Absorbent nanocrystalline-silver alginate vs Manuka honey vs paraffin tulle; DFU [59].	31 randomized (11/10/10). Computer randomization; sealed opaque envelopes; open-label treatment, blind wound evaluation. Three premature withdrawals; intention-to-treat and per-protocol analysis included.	Primary: 12-week complete healing, 81.8%/50%/40%; overall $p=0.267$. Silver vs tulle: HR 2.179 (95% CI 0.669–7.906).	F: small pilot and heterogeneous ulcers; the interval includes no difference. Area-reduction findings do not establish a complete-healing benefit or isolate silver from the dressing matrix.

Trial and product comparison	Randomization and analysis	Patient outcome and uncertainty	Claim-specific appraisal
Acticoat nanocrystalline-silver dressing vs non-silver dressings; acute DFU, without exposed tendon or osteomyelitis [17].	125 randomized (66/59); 118 analyzed (63/55), contributing 91/76 ulcers. Computer randomization stratified by infection/PAD; open label. Model accounts for multiple ulcers per participant.	Primary: ulcers healed at 12 weeks, 69% vs 75% ($p=0.49$); adjusted OR 0.92 (95% CI 0.26–3.22).	F: seven participants excluded after randomization; analysis required follow-up. Recruitment stopped short of planned ulcer target. No demonstrated benefit; the broad interval does not prove equivalence.
Nanordica Ag/Cu nanoparticle dressing vs Aquacel Ag+ Extra ionic-silver hydrofibre; mildly infected DFU [60].	30 randomized (17/13); sealed envelopes, participant blinding. All included in analyses; one control participant missed the final visit. Active phase 1 week; follow-up 2 weeks.	Safety-focused pilot. Area reduction: 43% vs 12% in Results (13% in abstract); overall decline comparison $p=0.151$. Baseline mean areas 475 vs 231 mm ² .	F, author upload: No power calculation or adjustment for multiplicity; Favorable visits and post hoc subgroups are exploratory. Various metal/matrix formulations and brief follow-up prohibit general superiority of nanoparticles.
Author-designated AgNP spray plus NPWT vs saline plus NPWT; post-revascularization diabetic wounds [18].	115 randomized (58/57); concealed computer block sequence; blinded evaluation. Per-protocol analysis: 50/50 after 8/7 losses; no intention-to-treat analysis.	Primary: six-month closure without graft, 74% vs 62%; difference 12 percentage points (95% CI –6.1 to 30.1), $p=0.198$.	Uncorrected author proof F: limitations in interpretation due to attrition, material characterization and secondary tables. No evidence for any closure advantage; secondary assertions need to be validated in final report.

F indicates that relevant full text sections have been read, and for the 2026 papers, the version uploaded by the author is mentioned. Effect estimates and confidence intervals represent reported values and not a combined estimate. DFU, diabetic foot ulcer; PAD, peripheral arterial disease; NPWT, negative-pressure wound therapy. This reflects a qualitative claim-based assessment and not any risk of bias scoring system. HR, hazard ratio; OR, odds ratio; CI, confidence interval. The percentages in Lafontaine study relate to ulcers, not participants.

Safety improvement and environmental performance.

Exposure of humans to models.

[48] have shown that the corona formation and interaction behavior depend on the type of natural polymer used for AgNP production; the reaction is different for whole blood and washed red blood cells. The biological matrix becomes important in addition to the nature of the coating. The biological activity depends on the capping agent's chemistry, as well as its dissolution in microorganisms and mammalian cells [61]. The comparison requires

compatibility testing on purified material in the correct matrix. concentrations of airborne silver of 0.35 and 1.35 $\mu\text{g}/\text{m}^3$ among two workers, with no significant changes in the observed health parameters [62]. This small and uncontrolled study cannot serve to establish a safety level or exclude any latent effects. It simply shows that exposure occurred in the workplace, while cell cultures and local dressing trials are done in other exposure environments.

Detoxification process is not universal through transformation

Decreased toxicity after sulfidation in various experimental models [63]. In conjunction with studies involving corona-induced transformation, this means that chemical speciation is fundamental to exposure assessment beyond the measurement of total silver. Protection at one degree of sulfidation does not imply protection at another degree.

The degree of sulfidation was consequential in the 2026 study by [64], Mild sulfidation of PVP-coated particles increased dissolution and mammalian-cell cytotoxicity, whereas more extensive sulfidation reduced the response. Increased hazard was associated with silver release, lipid peroxidation and evidence of ferroptosis. This nonmonotonic response requires the label sulfidized to be qualified by transformation extent, surface structure and test medium. The experiment establishes a controlled in vitro response; it does not measure environmental prevalence or population impact. The design targets are different: effective release during use and reduced bioavailability at disposal. Rapid antimicrobial release may not be compatible with the need for persistence or repeated exposure. Such trade-offs would be missed by characterisation of pristine particles, but can be identified by tracking silver mass, speciation and performance through storage, use and disposal.

Sustainability requires a defined comparison

The environmental performance is dependent on the functional unit, yield, energy, purification and waste included in the comparison. In a techno-economic and cradle-to-gate life-cycle assessment, [21] compared three biologically derived reduction pathways to a borohydride pathway. Their findings did not suggest that all natural extracts had a common benefit: the green-tea pathway did poorly in many comparisons because of its low yield and resource requirements. These are modelled process impacts based on stated assumptions, not direct measurements of toxicity to a treated

patient. Thus, a favorable life-cycle human-toxicity indicator should not substitute for a cytotoxicity or hemocompatibility test.

[22] investigated the prospective life-cycle performance of emerging chemical and biological routes. [65] coupled an ultrasound-intensified continuous process with process evaluation and life-cycle assessment. The second comparison was performed at lab scale. Taken together, these studies make the case for expanding evaluation of synthesis beyond the reagent list, while keeping the scale limits and model assumptions. A route which gives a less effective product in use may also compare well when comparisons are made per kilogram of silver product. An application-specific functional unit, such as a defined antimicrobial performance over a defined service period, would provide a more informative complementary comparison where evidence allows. Such a unit must be supported by actual service performance, not assumed equivalency.

Research priorities and a testable design framework

The design question must be defined by the application requirements. A localised antimicrobial coating needs a reproducible release range and host-tissue tolerance margin; an injected tumour-targeting concept needs distribution, clearance and tumour exposure evidence. These objectives could favour different diameters, coatings and dissolution behaviour. Figure 4 provides a proposed set of decision points for evidence progression; these are not regulatory approval criteria.

The first priority is controlled material families varying a limited set of targeted properties. Size series require release measurements. Comparisons of coatings need colloidal characterisation to distinguish between intended changes and differences due to exposure. Moreover, extract-based systems require input fingerprints, measured purification and independent synthesis batches, thus following the concept of reproducibility as discussed in Section 5.4 [3, 6, 52].

Exposure-resolved testing should couple time-matched core distributions, hydrodynamic state, and silver fractions with endpoints that discriminate alternative explanations. Three similarly affected optical dyes give less complementary evidence than a metabolic readout, viable-cell count and cell-free interference experiment. These measurements can be used in transport or dissolution models, provided that the models are independently validated [14, 15].

Comparisons need to be relevant to the purpose for which biological comparison is made: matrixes, biofilms, or reuse environments for antimicrobial products, along with nonmalignant controls whose growth conditions are compatible with the exposure of interest for tumor studies. Time-dependent analysis is required to show suppression of the resistance.

Prespecification of patient endpoints and formulation details are required for clinical evaluation [16, 18, 55].

Each possible enhancement involves an additional outcome that needs to be quantified. Decreased core size reduces specific surface area relative to weight but could increase release or cause tissue damage. Highly-bound coatings would decrease non-specific interactions while affecting exposure to the antibiotics; more purification would enhance interpretation while increasing energy consumption and waste production. Table 7 illustrates this by formulating a question corresponding to each gap, together with a discriminating experiment and a quantifiable outcome. It helps to conduct experiments without rating their quality numerically.

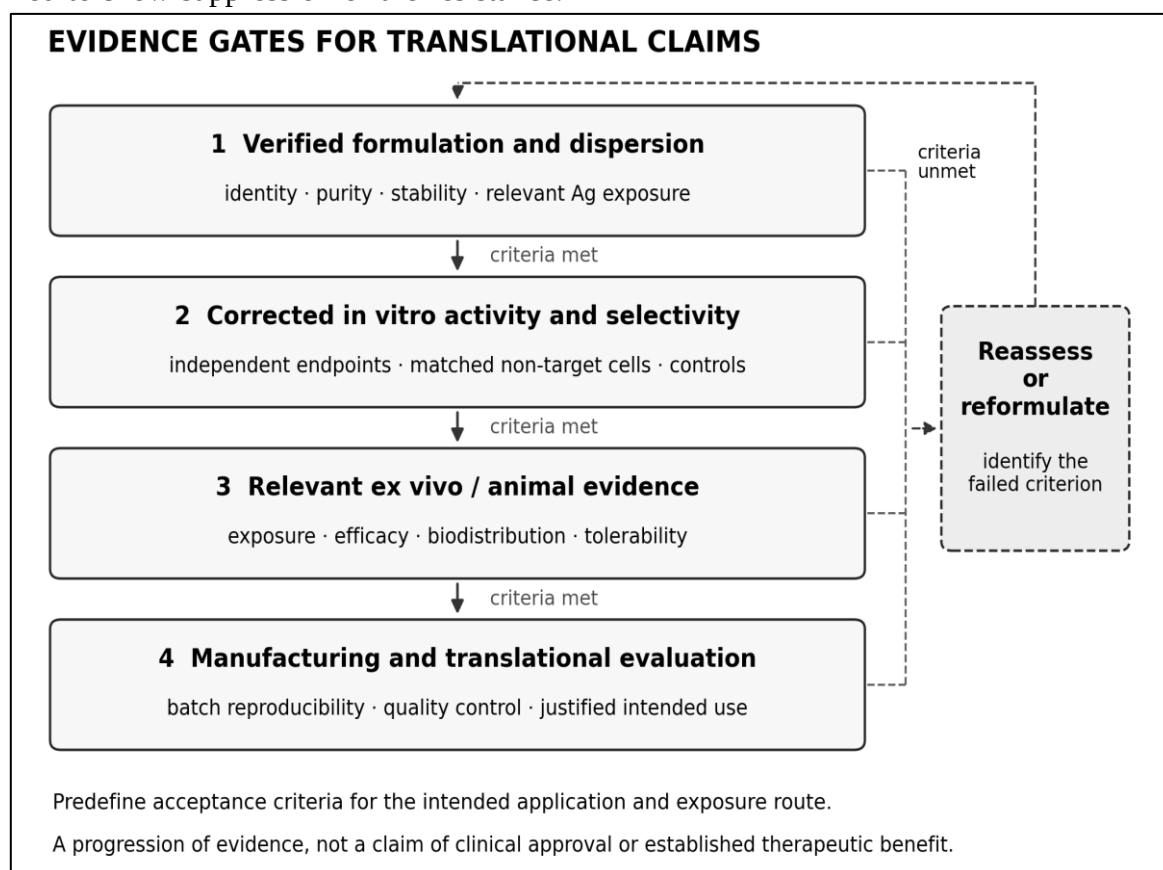


Figure 4. AgNP translation evidence flowchart. Progression requires application-defined criteria for each of the following steps: formulation validation; assay-corrected in vitro activity and selectivity; relevant ex vivo or animal evidence, where appropriate; and reproducible manufacturing and translational evaluation. The failure of any of these criteria results in re-evaluation and reformulation, and re-validation of the impacted steps. This conceptual decision tree is not meant to represent regulatory approval or clinical utility of an AgNP formulation.

Table 7. Priorities for research resulting from unanswered questions on synthesis–exposure–response relationships.

Unresolved question	Discriminating study	Required output	What would remain unproven
Does core size explain a response?	Related size series with surface measurements, release kinetics and multiple dose metrics	Size–response relation conditional on measured exposure	Universality beyond the tested chemistry and model [3, 13].
Can an extract route be reproduced?	Input fingerprinting, purification recovery and independently synthesized batches	Variance in material identity and a validated biological endpoint	Safety only because of the biological source [6, 52].
Does a coating improve the useful window?	Compare efficacy and host responses under relevant medium and exposure conditions	Matched exposure–response curves and uncertainty	Benefits in clinical practice by water stability or surface charge only [45, 48].
Does controlled release retain activity?	Time-resolved release with repeated-use, biofilm or serial-exposure tests	Activity and susceptibility linked to release history	Prevention of resistance in non-assayed systems or regimens [20, 55].
Is transformation protective?	Graded transformation with surface/speciation and target-specific assays	A transformation–response map rather than a binary label	Universal detoxification following sulfidation [63, 64].
Is a route environmentally preferable?	Life-cycle and process comparison with yield, scale and functional-unit sensitivity	Impacts per verified quantity and, where possible, per measured service performance	Biocompatibility or competitive edge from laboratory LCA only [21, 65].

The agenda is an attempt to integrate the findings. The success criterion must be predetermined for each application, not determined by the most powerful outcome.

CONCLUSIONS

Explanation of a biological difference through synthesis demands that there must be proven process alteration as well as measured relationship between the produced substance and delivered exposure or response. Geometry, surface chemistry, residual materials and transformation may all change at the same time. Interpretation of the measured response must be made based on those coupled changes.

These comparisons do not provide a universal ranking of activities. Geometry-dependent effects are dose-dependent and context-dependent biologically; particle-mediated and extracellular ion-mediated delivery can be different, and coronas and sulfidation can alter the effect one way or another. The antimicrobial effect needs to be evaluated along with the host damage and

adaptation, and tumor selectivity is dependent on comparable tests. The wound experiments are tests of specific formulations without determining the general relationship between synthesis and efficacy.

Future research should include comparisons of various materials, determination of exposure during testing, and repeatability across multiple synthesis processes. Such studies will be able to distinguish between persistent material properties and effects limited to only one synthesis or one test. The effectiveness in the application, biological compatibility, and environmental performance of materials each needs its own evidence.

Data availability

No new experimental data set has been used to perform this review. Supplementary search strings, number of hits and selection records at

the PMID level can be found in Appendix A. Quantitative results have been reported from the original references. Normalized plots of Figure 2 are fully described by Equations 1 and 2, along with the reference diameter mentioned above; all other figures are theoretical.

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