



Review Article

Open Access

Biocompatibility Assessment of Gold Nanoparticle Conjugates for Diagnostic Imaging

Ngo M^{1*}, Ngo H² and Dang D³

¹Department of Biomedical Research, Yangon Institute of Nanotechnology, Myanmar Scopus Author ID: 57887777971 Email: minh.ngo@yangon.edu
ORCID: <https://orcid.org/5432-0929-1141-6567>

²Department of Health Sciences, Ho Chi Minh City Center for Nanotechnology Research, Cambodia Scopus Author ID: 57013238022 Email: hai.ngo@ho.edu
ORCID: <https://orcid.org/8249-1158-5350-8073>

³Department of Health Sciences, Yangon Institute of Nanotechnology, Cambodia Scopus Author ID: 57384219640
Email: duc.dang@yangon.edu
ORCID: <https://orcid.org/9601-2506-2865-8721>

***Correspondence to:** Minh Ngo, Department of Biomedical Research, Yangon Institute of Nanotechnology, Myanmar Scopus Author ID: 57887777971 Email: minh.ngo@yangon.edu

Received: 29 December 2025; **Accepted:** 6 March 2026; **Published:** 13 May 2026

Copyright: © 2026 MN, et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Gold nanoparticles combine chemical inertness with size- and shape-tunable optical and radiographic properties, making them an attractive scaffold for contrast agents and molecular probes across computed tomography, positron emission tomography, and photoacoustic and optical imaging. Yet biocompatibility -- cytotoxicity, immune recognition, biodistribution, and eventual clearance -- is not a fixed property of gold nanoparticles as a class but a formulation-specific outcome shaped by core size, shape, surface charge, ligand chemistry, and even surface ligand chirality. This review synthesizes the literature on how these physicochemical variables determine cellular and systemic toxicity, how the protein corona that forms in vivo actively redefines a conjugate's biological identity, and what each major diagnostic imaging modality demands of a conjugate's biocompatibility profile. We give particular attention to the tension between ultrasmall, renal-clearable formulations designed to minimize long-term tissue retention and evidence that the same small size range can carry the greatest reported cytotoxicity in kidney cells. Read together, the evidence supports gold nanoparticle conjugates as a materially sound diagnostic imaging platform without a fundamental biocompatibility barrier, while leaving matched in vitro-to-in vivo validation, in vivo protein corona characterization, and long-term retention data on clinically representative conjugates as an open research agenda.

Keywords: gold nanoparticles; biocompatibility; diagnostic imaging; protein corona; nanotoxicology

1. Introduction

Gold has an odd distinction in nanomedicine: it is at once the most chemically inert metal used in any nanoparticle platform and the one whose biological fate turns out to be surprisingly hard to pin down. Colloidal gold's optical and electronic properties depend almost entirely on particle size and shape rather than on chemical reactivity, which is precisely what makes gold nanoparticles (AuNPs) so attractive as imaging labels -- their signal can be tuned by physics rather than by chemistry, while the metal core itself resists oxidation, dissolution, and the kind of ionic leaching that complicates the safety profile of many other inorganic nanomaterials. That combination has driven three decades of work exploring gold nanoparticle conjugates as contrast agents and molecular probes for computed tomography (CT), positron emission tomography (PET), photoacoustic imaging, and optical and ophthalmic imaging modalities [1,2].

But "chemically inert" is not the same thing as "biologically inert," and the diagnostic-imaging literature on gold nanoparticles has, over the past fifteen years, increasingly had to reckon with a biocompatibility picture that is considerably more nuanced than early enthusiasm suggested. The same physicochemical parameters that make a gold nanoparticle a useful imaging label -- core size, surface ligand chemistry, net surface charge, and aggregation state -- are exactly the parameters that govern how it is recognized by serum proteins, taken up by cells, distributed among organs, and ultimately cleared or retained by the body [3,4]. A citrate-capped 13 nm sphere and a PEGylated 60 nm sphere are, from a biological standpoint, essentially different materials, even though both are correctly described as "gold nanoparticles" in a paper's title.

This review synthesizes what the literature actually reports about the biocompatibility of gold nanoparticle conjugates intended for diagnostic imaging, organized around five threads: the physicochemical determinants of biological interaction (size, shape, surface charge, and ligand chemistry); the evidence on cellular and systemic toxicity from *in vitro* and *in vivo* studies; the protein corona as the biological interface that actually determines a nanoparticle's fate; the specific imaging modalities in which gold nanoparticle conjugates are being developed and what those applications demand of biocompatibility; and the renal clearance and long-term

biodistribution questions that remain the field's most persistent open problem. Our aim is not to argue that gold nanoparticles are safe or unsafe in any blanket sense -- the literature does not support either claim -- but to lay out, with the specificity the primary literature actually provides, what is known, what is contested, and what remains genuinely open for a material that is now approaching real diagnostic use rather than remaining a bench curiosity.

It is worth being explicit about scope from the outset. We are treating "biocompatibility" broadly, to include acute and chronic cytotoxicity, immune recognition, biodistribution and organ retention, and the eventual clearance pathway, because all four have been treated as biocompatibility endpoints in the imaging literature we surveyed, and because a nanoparticle that is non-toxic acutely but retained indefinitely in the liver raises a different set of regulatory and clinical questions than one that clears renally within days. We also want to flag, plainly, where the evidence is thinner than review articles sometimes imply: most of what follows comes from rodent studies and cell culture, not from large-animal or human pharmacokinetic data, and a meaningful fraction of the size- and shape-dependence literature was generated using nanoparticle libraries synthesized specifically to probe those variables rather than the exact imaging conjugates now moving toward translation.

2. Gold Nanoparticle Synthesis and the Physicochemical Variables That Matter

Most gold nanoparticles used in imaging research trace back to one of a small number of wet-chemical synthesis routes -- citrate reduction for spherical cores in the 10-100 nm range, seed-mediated growth for anisotropic shapes such as rods and stars, and thiol-based ligand exchange for surface functionalization after core synthesis -- and the resulting particles are then conjugated to imaging payloads (radiolabels, fluorophores, or simply left as the bare metal core, which is itself the contrast mechanism for CT and photoacoustic applications) and to targeting or stabilizing ligands such as polyethylene glycol (PEG), peptides, or antibodies. What matters for biocompatibility is less the synthesis route itself than the resulting physicochemical identity of the finished conjugate: hydrodynamic diameter (which is larger than the metal core diameter once ligands and any protein

layer are accounted for), surface charge (zeta potential), surface ligand density and chemistry, and aggregation state in physiological buffers and serum.

Size is probably the single most consistently reported determinant of biological behavior across the studies surveyed here, though "consistently reported" does not mean the relationship is simple or monotonic. Zhang et al. showed that PEG-coated gold nanoparticles of different core sizes, synthesized with the same PEGylation chemistry and evaluated in tumor-xenografted mice, differed substantially in colloidal stability and in vivo biodistribution depending on both particle size and the specific anchoring chemistry used to attach the PEG layer, with the anchoring ligand mattering at least as much as size alone in determining how much gold accumulated in liver and spleen versus remaining available to reach the tumor [5]. Separately, in a radiosensitization context rather than a pure imaging context, Zhang et al. tested PEG-coated gold nanoparticles across a size range and found that the biological effect (in that case, radiation dose enhancement in tumor-bearing mice) did not scale simply with size, underscoring that "smaller is more bioavailable" and "smaller is more effective" are not the same claim and should not be conflated when evaluating a candidate imaging conjugate [6]. Ligand composition tells a similar story: Liu et al. found that the specific mixture of PEG chain lengths and terminal chemistries anchored to a gold core changed the particle's in vivo fate -- its circulation behavior and its distribution among organs -- even holding core size constant, which is a useful reminder that "PEGylated" is a category, not a single well-defined surface [7].

Surface charge is the second major axis. Wang et al.'s comparison of gold nanoclusters bearing different surface charges found that charge state shaped both the long-term biodistribution pattern and the toxicity profile observed over extended in vivo follow-up, with charge interacting with the clusters' behavior in the context of cancer radiation therapy applications specifically [4]. This pattern -- cationic surfaces generally associating with greater nonspecific cellular uptake and, frequently, greater cytotoxicity relative to anionic or neutral surfaces of comparable size -- recurs often enough across the broader nanotoxicology literature that it is treated as close to a design rule by most groups developing new gold nanoparticle conjugates, even

though the magnitude of the charge effect varies considerably with core size, ligand chemistry, and the specific cell type or organ being examined.

A third, less frequently discussed variable is chirality of the surface ligand itself. Tang et al. demonstrated that reversing the chirality of the surface ligand shell on cationic gold nanoclusters -- keeping the same chemical composition but using the mirror-image ligand -- measurably improved both the biosafety profile and the pharmacokinetics of the resulting nanoclusters relative to the original chirality [8]. This is a striking result because it shows that biocompatibility is not fully determined by the coarse physicochemical descriptors (size, charge, hydrophilicity) that dominate most of the field's design heuristics; stereochemistry at the ligand-water interface can matter too, presumably because it changes how efficiently specific serum proteins recognize and bind the surface. We return to this point in the section on the protein corona, because ligand chirality is best understood as acting through corona composition rather than as an independent toxicity mechanism.

3. Cellular and Systemic Toxicity: Evidence from In Vitro and In Vivo Studies

The foundational finding that shaped much of the subsequent literature is that gold nanoparticle toxicity is strongly formulation-dependent rather than being a fixed property of "gold nanoparticles" as a class. Simpson et al. showed that monolayer-protected gold clusters that displayed unexpected toxicity in initial testing had that toxicity essentially eliminated by a PEG-thiol place-exchange reaction that altered the surface ligand shell without changing the metal core at all [9]. The practical implication is one that recurs throughout this literature: an observed toxicity result should be read as a statement about a specific formulation, not as a statement about gold nanoparticles in general, and comparing toxicity data across papers that use different capping ligands, even at nominally similar core sizes, requires real caution.

Direct evidence on renal cells is particularly relevant for diagnostic conjugates, since renal clearance (or the lack of it) is one of the primary fates any injected imaging agent will encounter. Enea and colleagues examined how gold nanoparticles of two sizes (13 nm and 60 nm), two shapes (spheres and stars), and two capping chemistries (citrate and mercaptoundecanoic acid,

MUA) affected human proximal tubular kidney cells (HK-2) after 24 hours of exposure [10]. The 13 nm MUA-capped nanospheres were the most toxic formulation tested, reducing cellular metabolic activity (by MTT assay) by up to 44% relative to untreated controls, and reducing lysosomal integrity (by neutral red assay) by roughly 37% for both the citrate- and MUA-capped 13 nm nanospheres; the same study found evidence of mitochondrial dysfunction, increased reactive oxygen species production, and activation of both extrinsic and intrinsic apoptotic pathways (caspase-8 and caspase-9) specifically in the smaller nanospheres, while the 60 nm particles were substantially less toxic across every metric examined. This is a genuinely useful data point for imaging-conjugate design because it isolates size as a variable while holding shape and capping chemistry approximately constant across comparisons, and because renal proximal tubule cells are directly relevant to the clearance pathway that ultrasmall gold conjugates are specifically designed to exploit -- a tension we return to below.

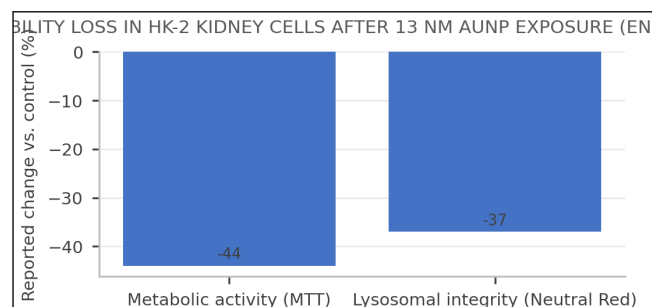


Figure 1: Reduction in human kidney (HK-2) cell viability after 24-hour exposure to 13 nm mercaptoundecanoic-acid-capped gold nanospheres, by assay, relative to untreated controls -- the size range most favorable for renal clearance was also the most cytotoxic in this study, illustrating the tension between clearance-oriented and toxicity-oriented formulation design discussed in this review [10].

Systemic, whole-animal toxicity and clearance data tell a broadly reassuring but formulation-sensitive story. Simpson et al. examined the *in vivo* toxicity, biodistribution, and clearance of glutathione-coated gold nanoparticles and found a biodistribution and clearance pattern consistent with the small, hydrophilic, zwitterionic character of the glutathione coating, without evidence of the severe organ toxicity that some earlier, less carefully controlled studies of gold nanoparticles had reported [11]. Comparable formulation sensitivity shows up in Zhang et al.'s comparison of three classes of PEGylated gold nanoparticles -- solid PEGylated spheres, PEGylated

aggregates formed through two different cross-linking chemistries (PTMP and TTMP), and a hollow-shell construction -- evaluated for both *in vitro* macrophage uptake and *in vivo* biodistribution in the same study [3]. All three classes showed rapid blood clearance, with most material essentially cleared from circulation within an hour of injection, but the aggregate formulations showed significantly higher liver accumulation and shorter effective circulation time than the solid PEGylated spheres despite showing similarly low macrophage uptake in the *in vitro* assay -- a mismatch the authors highlight as a caution against relying on *in vitro* uptake screens as a stand-in for *in vivo* biodistribution behavior. That disconnect between *in vitro* and *in vivo* readouts is, in our view, one of the more practically important findings in the biocompatibility literature for anyone screening candidate imaging conjugates, because it means a formulation that looks favorable in a cell-culture assay cannot be assumed to behave the same way once it encounters the full complexity of serum, the reticuloendothelial system, and organ-level clearance mechanisms.

A protein-templated approach offers a somewhat different route to biocompatibility. Yang et al. used a human endogenous protein as a biomimetic scaffold to synthesize paramagnetic gold nanostructures for tumor bimodal imaging, exploiting the idea that building the imaging conjugate around a protein the body already recognizes as "self" might reduce the immune and clearance complications that a purely synthetic ligand shell can introduce [12]. This general strategy -- biomimetic or protein-templated gold nanostructure synthesis -- represents one of several attempts in the recent literature to engineer biocompatibility in from the start of synthesis rather than optimizing it post hoc through ligand exchange, and it connects directly to the broader question of how the body's own protein machinery ends up defining a nanoparticle's biological identity, which is the subject of the next section.

4. The Protein Corona and Biological Identity

Within seconds of entering blood or interstitial fluid, any gold nanoparticle conjugate is coated by a layer of adsorbed serum proteins -- the protein corona -- and it is this corona, not the nanoparticle's bare synthetic surface, that cells, immune receptors, and clearance organs actually encounter. Charbgoon et al. have argued that understanding the protein corona is close to a

prerequisite for any gold nanoparticle formulation intended for clinical use, because the corona effectively rewrites the nanoparticle's surface identity after the fact, regardless of how carefully that surface was engineered during synthesis. Corona composition depends on core size and shape even when the underlying synthetic surface chemistry is held constant: García-Álvarez et al. examined *in vivo* protein corona formation directly (rather than inferring corona composition from *in vitro* serum incubation, which does not always reproduce what happens in a living animal) and found that gold nanoparticle size and shape measurably changed which proteins dominated the corona and in what relative abundance [13]. This *in vivo*-versus-*in vitro* distinction matters a great deal for biocompatibility assessment specifically, because a corona profile generated by simply incubating a nanoparticle in serum on the bench can differ substantially from the corona that forms once the particle is circulating through an intact vasculature and encountering the full dynamic protein environment of a living organism, including proteins produced locally by the liver, spleen, and vascular endothelium rather than merely present in stored serum.

The corona is not a passive coating; it actively participates in determining the therapeutic and diagnostic behavior of the particle. Cui et al. showed that the composition of the protein corona could be deliberately guided through surface modulation with low-molecular-weight PEG, and that this corona-guided approach improved tumor targeting outcomes relative to conventional PEGylation strategies, effectively using corona engineering as a design tool rather than treating the corona purely as a confound to be minimized [14]. This reframes the biocompatibility question in a useful way: rather than asking only "does this surface chemistry avoid triggering an adverse corona," some recent work asks "can we design the surface chemistry to recruit a favorable corona" -- for instance, one enriched in proteins associated with reduced macrophage recognition, or one that supports longer circulation and more efficient tumor or target-tissue accumulation.

The corona also intersects directly with biocompatibility in the more traditional sense of reducing adverse biological responses. Landgraf et al. demonstrated that a plasma protein corona could enhance the biocompatibility of Au@Fe₃O₄ Janus particles (hybrid gold-iron oxide constructs relevant to multimodal

imaging), showing that the corona was not merely an inevitable complication to be engineered around but could, under the right circumstances, function protectively [15]. Taken together with the chirality result discussed above -- where reversing ligand chirality improved biosafety and pharmacokinetics, plausibly by changing which serum proteins bound the surface and how tightly [8] -- the corona literature supports a fairly specific conclusion: biocompatibility for gold nanoparticle conjugates is not a fixed property that can be engineered once at the level of bare-surface chemistry and then assumed to hold; it is an emergent property of the corona that forms once the conjugate meets a living biological system, and predicting that corona from synthetic parameters alone remains an imperfect science.

5. Gold Nanoparticle Conjugates Across Diagnostic Imaging Modalities

Gold's biocompatibility profile cannot be evaluated in the abstract; it has to be evaluated against the specific dose, conjugate chemistry, and administration route that a given imaging application requires, and those requirements differ substantially across modalities.

Computed tomography is historically the most direct application of gold nanoparticles as imaging agents, because gold's high atomic number and electron density give it strong intrinsic X-ray attenuation, allowing bare or minimally functionalized gold cores to serve directly as the contrast mechanism without a separate reporter payload. Cole and colleagues' review of gold nanoparticles as CT contrast agents lays out why this matters for biocompatibility specifically: because gold's attenuation efficiency per unit mass is favorable relative to conventional iodinated contrast media, CT applications can in principle use lower absolute doses of metal to achieve equivalent contrast, which is directly relevant to minimizing whatever dose-dependent toxicity risk the formulation carries [16]. This advantage has been extended toward spectral and photon-counting CT, which exploits gold's distinct K-edge to distinguish gold-labeled structures from other tissue and contrast signals; Si-Mohamed et al. used photon-counting CT for *in vivo* molecular K-edge imaging of atherosclerotic plaque, and Moghiseh et al. specifically labeled circulating monocytes with gold nanoparticles for spectral photon-counting CT detection of atherosclerosis in a preclinical model, both

applications in which the gold conjugate needs to survive circulation, reach and label a specific cell population or tissue, and do so without provoking the kind of immune activation that would itself confound an atherosclerosis-imaging readout [17,18]. The broader literature on X-ray CT contrast agents, reviewed comprehensively by Lusic and Grinstaff, situates gold nanoparticles within a wider landscape of iodinated, gadolinium-based, and nanoparticulate CT contrast strategies, and underscores that biocompatibility and pharmacokinetic clearance profile, not just attenuation efficiency, are what ultimately determine whether a candidate CT agent is viable for repeated or diagnostic use [19].

Table 1: Representative gold nanoparticle conjugate applications across diagnostic imaging modalities, with the biocompatibility consideration each study identifies as most consequential for that application.

Modality	Representative Study	Conjugate / Strategy	Principal Biocompatibility Consideration
X-ray CT	Cole et al. [16]	Bare or minimally coated Au core as direct attenuator	Favorable attenuation-per-mass vs. iodinated media allows lower administered metal dose
Spectral / photon-counting CT	Moghiseh et al.; Si-Mohamed et al. [18,17]	AuNP-labeled circulating monocytes; K-edge molecular imaging	Labeled cells must survive circulation without immune activation confounding the readout
PET	Chen et al. [20]	Ultrasmall, renal-clearable radiolabeled AuNPs	Renal filtration threshold size range coincides with greatest reported kidney-cell cytotoxicity
Photoacoustic imaging	Li & Chen; Barlow & Kim [22,23]	Anisotropic (rod/star/cage) plasmonic AuNPs	Shape-optimized synthesis often requires capping chemistries (e.g., CTAB) needing post-hoc exchange for biocompatible ligands
Ophthalmic / retinal optical imaging	Chen et al.; Park et al. [25,26]	Locally (intraocular/topical) delivered AuNPs	Local delivery route limits systemic exposure relative to intravenous administration
Multimodal molecular imaging	Bouché et al.; Mieszawska et al. [1,2]	AuNP core with combined CT/fluorescent/radiolabel payloads	Each added functional component independently shifts surface charge, corona composition, and biodistribution

PET and other radiolabeled-tracer applications place different demands on the conjugate, because here the gold core is typically functioning as a nanoparticulate

scaffold for a radioisotope payload rather than as the imaging signal itself, and the biocompatibility question becomes at least as much about the pharmacokinetics of the intact conjugate (does it clear efficiently, does the radiolabel stay attached, does the biodistribution pattern match what the target application needs) as about acute cytotoxicity. Chen et al.'s dynamic PET imaging study of renal-clearable gold nanoparticles is a useful example precisely because it ties the imaging application directly to the clearance question: the ultrasmall AuNPs used were specifically designed to be small enough to undergo renal filtration, and PET imaging was used to directly visualize that clearance process in real time in living animals, rather than inferring it indirectly from tissue harvesting at fixed endpoints [20]. Jiang et al.'s broader review of nanomedicines for renal imaging and treatment situates this renal-clearance strategy within the wider field, making the case that renal-clearable nanoparticle platforms -- gold included -- represent a deliberate biocompatibility strategy in their own right: minimize long-term tissue retention by designing the particle to be small enough to exit through the kidneys rather than accumulate in the liver and spleen the way most larger nanoparticles do [21].

Optical and photoacoustic imaging applications exploit gold's plasmonic properties -- the strong, size- and shape-tunable absorption and scattering that arise from the collective oscillation of conduction electrons at the nanoparticle surface -- and here biocompatibility questions intersect closely with the shape-dependence findings discussed earlier, because anisotropic shapes (rods, stars, cages) that are often preferred for their tunable near-infrared absorption tend to require different, sometimes more complex, surface capping chemistries (such as cetyltrimethylammonium bromide, CTAB, for gold nanorod synthesis) than simple citrate-capped spheres, and those capping chemistries carry their own, sometimes worse, toxicity profiles unless subsequently exchanged for a more biocompatible coating. Li and Chen's review of gold nanoparticles for photoacoustic imaging and Barlow and Kim's more recent survey of next-generation gold nanomaterials for photoacoustic imaging both track this tension between optimizing a nanostructure's optical properties for imaging performance and ensuring the resulting surface chemistry is compatible with systemic administration [22,23]. Liu et al.'s review of gold

nanoparticle theranostics with an emphasis on photoacoustic imaging and photothermal therapy makes a related point: many of the same anisotropic gold nanostructures optimized for photoacoustic contrast are also being developed as photothermal therapeutic agents, meaning the biocompatibility bar for these constructs is set not just by diagnostic dosing but by the higher, repeated, or therapeutic-intent dosing that a combined diagnostic-and-therapeutic ("theranostic") application implies [24]. Ophthalmic applications illustrate a route around some of these systemic concerns: Chen et al.'s review of gold nanoparticles for ophthalmic imaging and Park et al.'s work on gold nanoparticles for retinal molecular optical imaging both discuss local, intraocular or topical delivery routes that can achieve high local gold concentration for imaging contrast while limiting the systemic exposure that drives most of the biodistribution and long-term retention concerns raised elsewhere in this review [25,26]. Bouché et al.'s broader review of molecular imaging with gold nanoparticles and Mieszawska et al.'s review of multifunctional gold nanoparticles for diagnosis and therapy both make the point that many contemporary imaging conjugates are explicitly multimodal -- combining, for instance, CT contrast with a fluorescent or radiolabeled reporter on the same gold scaffold -- which compounds rather than simplifies the biocompatibility assessment, since each added functional component (targeting ligand, secondary label, linker chemistry) is itself a variable that can shift surface charge, corona composition, and ultimate biodistribution [1,2].

6. Renal Clearance and Long-Term Biodistribution

If there is a single biocompatibility problem that recurs across essentially every application discussed above, it is what happens to the gold after the imaging study is complete. Gold nanoparticles are not metabolized or biodegraded the way many organic or lipid-based nanocarriers are; the metal core is, for practical purposes, permanent, which means the only mechanisms available for removing gold from the body are excretion (predominantly renal, for sufficiently small particles) or indefinite sequestration, typically in the liver and spleen via the reticuloendothelial system.

This is why the ultrasmall, renal-clearable design strategy discussed above in the PET context has

attracted so much attention as a biocompatibility solution rather than merely an imaging optimization. Chen et al.'s direct PET visualization of renal clearance for ultrasmall gold nanoparticles provides real-time evidence that particles engineered to fall below the renal filtration size threshold do, in fact, clear through the kidneys on a clinically reasonable timescale rather than accumulating [20], and Jiang et al.'s review situates this strategy within the broader push toward renal-clearable nanomedicine platforms specifically because avoiding indefinite hepatic and splenic retention is treated, across the field, as one of the more important unresolved biocompatibility goals for any metal-based nanoparticle intended for repeated or diagnostic-scale human use [21]. But engineering a particle small enough to clear renally reopens the toxicity question raised by Enea et al.'s kidney-cell data: the same small size range that favors renal filtration is also, in that study, the size range associated with the greatest cytotoxicity to renal proximal tubule cells specifically [10]. This is not necessarily a contradiction -- transient passage through the kidney during clearance is a different exposure regime than sustained cell-culture incubation, and the surface chemistry used for imaging-grade renal-clearable conjugates (typically zwitterionic or glutathione-type coatings) differs from the citrate and MUA coatings tested in the kidney-cell toxicity study -- but it does mean that renal clearance should not be treated as an automatically safe alternative to hepatic retention without formulation-specific toxicity testing on the actual conjugate intended for use, at the actual size and surface chemistry planned for administration.

For particles that are not small enough to clear renally, the ligand and PEGylation chemistry questions raised earlier become the primary lever for reducing long-term retention. Zhang et al.'s and Liu et al.'s biodistribution studies both point toward the same general conclusion from slightly different angles: the specific PEG anchoring chemistry and ligand composition, not just the presence of a PEG coating per se, determines how much gold ends up sequestered in liver and spleen versus remaining in circulation long enough to reach its imaging target and subsequently be cleared [5,7]. Simpson et al.'s glutathione-coated particle data reinforces the same point from the clearance side, showing that a specifically chosen small, zwitterionic capping ligand shapes the clearance pathway favorably relative to less carefully optimized coatings [11]. None of this amounts to a fully

solved problem -- long-term (multi-month to multi-year) retention data in large animals or humans remains sparse across this entire literature -- but the direction of travel across the biodistribution and renal-clearance studies surveyed here is toward formulations explicitly engineered to minimize permanent tissue burden, rather than toward simply accepting hepatic and splenic retention as an unavoidable cost of using a non-biodegradable metal core.

7. Open Challenges

Several gaps recur across the literature synthesized here and deserve to be stated plainly rather than smoothed over. First, in vitro-to-in vivo translation remains unreliable: Zhang et al.'s direct comparison of macrophage uptake in vitro against biodistribution in vivo found no meaningful correlation between the two for several gold nanoparticle classes, which should give real pause to any biocompatibility conclusion drawn purely from cell-culture screening without in vivo confirmation [3]. Second, the protein corona is now understood to be central to biological identity, but predicting corona composition from synthetic surface parameters alone remains imperfect, and the in vivo corona can differ meaningfully from the corona measured after simple serum incubation on the bench, which means a meaningful share of published corona characterization may not reflect what actually happens after intravenous administration [13].

Third, size-dependent toxicity and size-dependent renal clearance point in tension with each other for the smallest, most clearance-favorable gold conjugates, and this tension has not been resolved by formulation-matched, dose-response studies that test the exact imaging-grade conjugate (not a generic citrate- or MUA-capped comparator) across the size range relevant to renal filtration [10,20]. Fourth, most of the biocompatibility evidence synthesized here comes from short-to-medium-term rodent studies; genuinely long-term retention, chronic low-dose toxicity, and large-animal or human pharmacokinetic data remain comparatively scarce relative to the volume of acute toxicity and short-term biodistribution literature the field has generated. Fifth, and more practically: as imaging conjugates become more elaborate -- combining targeting ligands, multiple imaging payloads, and engineered corona-modulating surface chemistry

on a single gold scaffold, as reviewed for molecular and multifunctional imaging applications [1,2] -- each additional functional component reopens the biocompatibility question rather than closing it, and comprehensive biocompatibility data on the fully assembled, clinically representative conjugate (rather than on the bare gold core or on a simplified model formulation) remains the exception rather than the norm across the papers surveyed here.

We do not think any of these gaps are disqualifying for gold nanoparticle conjugates as a diagnostic imaging platform; the consistency with which independent groups, working across CT, PET, and optical/photoacoustic modalities, have converged on similar design principles -- favor small, well-characterized, zwitterionic or carefully anchored PEG surface chemistries; characterize the corona in vivo rather than only in vitro; and treat renal clearance as a design goal rather than an afterthought -- suggests the underlying materials science is sound. But translating that consensus into fully assembled, clinically representative conjugates with matched long-term safety data remains, honestly, unfinished work.

8. Conclusion

The literature reviewed here supports a specific, moderately optimistic but clearly qualified claim about gold nanoparticle conjugates for diagnostic imaging. Gold's chemical inertness and tunable optical and radiographic properties make it a genuinely versatile scaffold across CT, PET, photoacoustic, and optical imaging modalities [16,22,20,25], and the accumulated toxicology and biodistribution literature does not point to a fundamental, unavoidable biocompatibility barrier to that use. What it does point to, repeatedly, is that biocompatibility for any specific gold nanoparticle conjugate is a formulation-level property, not a class-level one: size, shape, surface charge, ligand chemistry, and the corona that forms once the conjugate meets a living organism all interact to determine whether a given construct behaves like the well-tolerated, efficiently cleared formulations reported by several of the groups discussed here, or like the more toxic, more persistently retained formulations reported by others, sometimes using nominally similar core sizes [9,4,10].

Making further progress on this question, in our reading, depends less on new synthesis chemistry and more on

closing the specific evidentiary gaps identified above: matched in vitro and in vivo testing on the same conjugate rather than on model formulations, in vivo rather than purely in vitro protein corona characterization, size-and-clearance studies that use the actual imaging-grade surface chemistry rather than generic comparator coatings, and longer-duration retention and toxicity data than the field has so far generated at scale. None of these are reasons to abandon gold nanoparticle conjugates as a diagnostic imaging strategy; they are, taken together, closer to a specific research agenda for the next stage of translating this platform from an extensively characterized preclinical material into a clinically deployed one.

9. References

1. Bouché, M., Hsu, J.C., Dong, Y.C., Kim, J., Taing, K., Cormode, D.P. (2020) Recent Advances in Molecular Imaging with Gold Nanoparticles. *Bioconjugate Chemistry*, 31(2): 303-314. DOI: [10.1021/acs.bioconjchem.9b00669](https://doi.org/10.1021/acs.bioconjchem.9b00669)
2. Mieszawska, A.J., Mulder, W.J., Fayad, Z.A., Cormode, D.P. (2013) Multifunctional Gold Nanoparticles for Diagnosis and Therapy of Disease. *Molecular Pharmaceutics*, 10(3): 831-847. DOI: [10.1021/mp3005885](https://doi.org/10.1021/mp3005885)
3. Zhang, Y., Liu, A.T., Cornejo, Y.R., Van Haute, D., Berlin, J.M. (2020) A Systematic Comparison of In Vitro Cell Uptake and In Vivo Biodistribution for Three Classes of Gold Nanoparticles with Saturated PEG Coatings. *PLoS One*, 15(7): e0234916. DOI: [10.1371/journal.pone.0234916](https://doi.org/10.1371/journal.pone.0234916)
4. Wang, J.Y., et al. (2016) Effects of Surface Charges of Gold Nanoclusters on Long-Term In Vivo Biodistribution, Toxicity, and Cancer Radiation Therapy. *International Journal of Nanomedicine*, 11: 3475-3485. DOI: [10.2147/IJN.S106073](https://doi.org/10.2147/IJN.S106073)
5. Zhang, G., Yang, Z., Lu, W., Zhang, R., Huang, Q., Tian, M., Li, L., Liang, D., Li, C. (2009) Influence of Anchoring Ligands and Particle Size on the Colloidal Stability and In Vivo Biodistribution of Polyethylene Glycol-Coated Gold Nanoparticles in Tumor-Xenografted Mice. *Biomaterials*, 30(10): 1928-1936. DOI: [10.1016/j.biomaterials.2008.12.038](https://doi.org/10.1016/j.biomaterials.2008.12.038)
6. Zhang, X.D., Wu, D., Shen, X., Chen, J., Sun, Y.M., Liu, P.X., Liang, X.J. (2012) Size-Dependent Radiosensitization of PEG-Coated Gold Nanoparticles for Cancer Radiation Therapy. *Biomaterials*, 33(27): 6408-6419. DOI: [10.1016/j.biomaterials.2012.05.047](https://doi.org/10.1016/j.biomaterials.2012.05.047)
7. Liu, X., Huang, N., Wang, H., Li, H., Jin, Q., Ji, J. (2013) The Effect of Ligand Composition on the In Vivo Fate of Multidentate Poly(ethylene Glycol) Modified Gold Nanoparticles. *Biomaterials*, 34(33): 8370-8381. DOI: [10.1016/j.biomaterials.2013.07.059](https://doi.org/10.1016/j.biomaterials.2013.07.059)
8. Tang, H., et al. (2021) Reversing the Chirality of Surface Ligands Can Improve the Biosafety and Pharmacokinetics of Cationic Gold Nanoclusters. *Angewandte Chemie International Edition*, 60(25): 13829-13834. DOI: [10.1002/anie.202101609](https://doi.org/10.1002/anie.202101609)
9. Simpson, C.A., et al. (2010) Unexpected Toxicity of Monolayer Protected Gold Clusters Eliminated by PEG-Thiol Place Exchange Reactions. *Chemical Research in Toxicology*, 23(10): 1608-1616. DOI: [10.1021/tx100209t](https://doi.org/10.1021/tx100209t)
10. Enea, M., et al. (2020) Gold Nanoparticles Induce Oxidative Stress and Apoptosis in Human Kidney Cells. *Nanomaterials*, 10(5): 995. DOI: [10.3390/nano10050995](https://doi.org/10.3390/nano10050995)
11. Simpson, C.A., Salleng, K.J., Cliffl, D.E., Feldheim, D.L. (2013) In Vivo Toxicity, Biodistribution, and Clearance of Glutathione-Coated Gold Nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*, 9(2): 257-263. DOI: [10.1016/j.nano.2012.06.002](https://doi.org/10.1016/j.nano.2012.06.002)
12. Yang, W., et al. (2018) A Human Endogenous Protein Exerts Multi-Role Biomimetic Chemistry in Synthesis of Paramagnetic Gold Nanostructures for Tumor Bimodal Imaging. *Biomaterials*, 161: 256-269. DOI: [10.1016/j.biomaterials.2018.01.050](https://doi.org/10.1016/j.biomaterials.2018.01.050)
13. García-Álvarez, R., Hadjidemetriou, M., Sánchez-Iglesias, A., Liz-Marzán, L.M., Kostarelos, K. (2018) In Vivo Formation of Protein Corona on Gold Nanoparticles. The Effect of Their Size and Shape. *Nanoscale*, 10(3): 1256-1264. DOI: [10.1039/c7nr08322j](https://doi.org/10.1039/c7nr08322j)
14. Cui, T., Ma, Y., Yang, J.Y., et al. (2021) Protein Corona-Guided Tumor Targeting Therapy via the Surface Modulation of Low Molecular Weight PEG. *Nanoscale*, 13(11): 5883-5891. DOI: [10.1039/d1nr00426c](https://doi.org/10.1039/d1nr00426c)
15. Landgraf, L., Christner, C., Storck, W., et al. (2015) A Plasma Protein Corona Enhances the Biocompatibility of Au@Fe₃O₄ Janus Particles. *Biomaterials*, 68: 77-88. DOI: [10.1016/j.biomaterials.2015.07.049](https://doi.org/10.1016/j.biomaterials.2015.07.049)
16. Cole, L.E., Ross, R.D., Tilley, J.M., Vargo-Gogola, T., Roeder, R.K. (2015) Gold nanoparticles as contrast agents in x-ray imaging and computed tomography. *Nanomedicine (Lond)*, 10(2): 321-341. DOI: [10.2217/nnm.14.171](https://doi.org/10.2217/nnm.14.171)
17. Si-Mohamed, S.A., Sigovan, M., Hsu, J.C., et al. (2021) In Vivo Molecular K-Edge Imaging of Atherosclerotic Plaque Using Photon-counting CT. *Radiology*, 300(1): 98-107. DOI: [10.1148/radiol.2021203968](https://doi.org/10.1148/radiol.2021203968)
18. Moghiseh, M., Searle, E., Dixit, D., Kim, J., Dong, Y.C., Cormode, D.P., Butler, A., Gieseg, S.P. (2023) Spectral Photon-Counting CT Imaging of Gold Nanoparticle Labelled Monocytes for Detection of Atherosclerosis: A Preclinical

- Study. Diagnostics, 13(3): 499. DOI: [10.3390/diagnostics13030499](https://doi.org/10.3390/diagnostics13030499)
19. Lusic, H., Grinstaff, M.W. (2013) X-ray-computed tomography contrast agents. *Chemical Reviews*, 113(3): 1641-1666. DOI: [10.1021/cr200358s](https://doi.org/10.1021/cr200358s)
 20. Chen, F., Goel, S., Hernandez, R., Graves, S.A., Shi, S., Nickles, R.J., Cai, W. (2016) Dynamic Positron Emission Tomography Imaging of Renal Clearable Gold Nanoparticles. *Small*, 12(20): 2775-2782. DOI: [10.1002/sml.201600194](https://doi.org/10.1002/sml.201600194)
 21. Jiang, D., Rosenkrans, Z.T., Ni, D., Lin, J., Huang, P., Cai, W. (2020) Nanomedicines for Renal Management: From Imaging to Treatment. *Accounts of Chemical Research*, 53(9): 1869-1880. DOI: [10.1021/acs.accounts.0c00323](https://doi.org/10.1021/acs.accounts.0c00323)
 22. Li, W., Chen, X. (2015) Gold Nanoparticles for Photoacoustic Imaging. *Nanomedicine (Lond)*, 10(2): 299-320. DOI: [10.2217/nnm.14.169](https://doi.org/10.2217/nnm.14.169)
 23. Barlow, B.R., Kim, J. (2025) Next Generation Gold Nanomaterials for Photoacoustic Imaging. *Nanomedicine (Lond)*, 20(12): 1479-1493. DOI: [10.1080/17435889.2025.2504330](https://doi.org/10.1080/17435889.2025.2504330)
 24. Liu, Y., Ma, W., Wang, J. (2018) Theranostics of Gold Nanoparticles with an Emphasis on Photoacoustic Imaging and Photothermal Therapy. *Current Pharmaceutical Design*, 24(23): 2719-2728. DOI: [10.2174/1381612824666180604112201](https://doi.org/10.2174/1381612824666180604112201)
 25. Chen, F., Si, P., de la Zerda, A., Jokerst, J.V., Myung, D. (2021) Gold Nanoparticles to Enhance Ophthalmic Imaging. *Biomaterials Science*, 9(2): 367-390. DOI: [10.1039/d0bm01063d](https://doi.org/10.1039/d0bm01063d)
 26. Park, S., Nguyen, V.P., Wang, X., Paulus, Y.M. (2024) Gold Nanoparticles for Retinal Molecular Optical Imaging. *International Journal of Molecular Sciences*, 25(17): 9315. DOI: [10.3390/ijms25179315](https://doi.org/10.3390/ijms25179315)

10. Declarations

Funding

This literature synthesis received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare that they have no competing interests relevant to the content of this article.

Data Availability Statement

This article is a literature synthesis and did not generate new experimental data. All findings discussed are drawn from the cited publications, which should be consulted directly for their underlying data and materials availability statements.

Ethical Approval

Not applicable. This review involved no human participants, animal subjects, or primary data collection by the authors.

Citation: Ngo M, Ngo H, Dang D. "Biocompatibility Assessment of Gold Nanoparticle Conjugates for Diagnostic Imaging." *J Nanotechnol Nanomed Res* (2026): 001-010 doi: [10.5281/zenodo.22849437](https://doi.org/10.5281/zenodo.22849437)