

Gold nanoparticles and angiogenesis: molecular mechanisms and biomedical applications

This article was published in the following Dove Press journal:
International Journal of Nanomedicine

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Abstract: Angiogenesis is the formation of new blood vessels from pre-existing vessels. It is a highly regulated process as determined by the interplay between pro-angiogenic and anti-angiogenic factors. Under certain conditions the balance between angiogenesis stimulators and inhibitors is altered, which results in a shift from physiological to pathological angiogenesis. Therefore, the goal of therapeutic targeting of angiogenic process is to normalize vasculature in target tissues by enhancing angiogenesis in disease conditions of reduced vascularity and blood flow, such as tissue ischemia, or alternatively to inhibit excessive and abnormal angiogenesis in disorders like cancer. Gold nanoparticles (AuNPs) are special particles that are generated by nanotechnology and composed of an inorganic core containing gold which is encircled by an organic monolayer. The ability of AuNPs to alter vasculature has captured recent attention in medical literature as potential therapeutic agents for the management of pathologic angiogenesis. This review provides an overview of the effects of AuNPs on angiogenesis and the molecular mechanisms and biomedical applications associated with their effects. In addition, the main synthesis methods, physical properties, uptake mechanisms, and toxicity of AuNPs are briefly summarized.

Keywords: gold nanoparticles, angiogenesis, molecular signaling, VEGF, uptake, toxicity

Angiogenesis: overview

Angiogenesis is the formation of new blood vessels from pre-existing vessels.¹⁻⁴ It is a highly regulated process in which the interplay between pro-angiogenic and anti-angiogenic factors mediates the transition of vascular endothelium through different stages of vascular growth. Angiogenesis is divided into multiple sequential phases of vascular development which are activation, progression, migration, differentiation, and maturation of endothelial cells.^{5,6} Under conditions of hypoxia, inflammation, or vascular injury; angiogenic signals stimulate quiescent endothelial cells to take an activated state.⁵⁻⁷ During the activation phase, vascular permeability is increased and pericytes detach away from endothelial cells through the effect of different angiogenic mediators. Activation of endothelial cells is followed by degradation of the extracellular matrix and subsequent migration of endothelial cells during the progression phase.^{2,8,9} Endothelial cell destabilization and migration is regularly mediated by angiopoietins (Angs), matrix metalloproteinases (MMPs), chymases, and heparanases which collectively enhance degradation of matrix molecules and facilitate formation of new vascular structures by the activated endothelial cells.^{5,6,10-12} Upon development of new blood vessels, maturation and stabilization of the new vessels take place through pericyte attachment and the resumption of the quiescent state of the endothelial

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cells.^{5,6} Angiogenesis is mediated by two major mechanisms; sprouting and intussusception (Figure 1).

Angiogenesis is controlled by multiple pro-angiogenic and anti-angiogenic factors. Angiogenic factors include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF- β), epidermal growth factor (EGF), placental growth factor (PIGF), cytokines (e.g. tumor necrosis factor-alpha (TNF- α), interleukin (IL)-8), Ang-1, Ang-2, and

angiogenin.^{13,14} Among the pro-angiogenic factors, VEGF-A is a major regulator of vascular growth. Anti-angiogenic factors include thrombospondin-1, angiostatin, endostatin, vasostatin, tumstatin, interferon- γ (IF- γ), glycosaminoglycan (heparins), α v integrin antagonists, β 1 integrin antagonists, anti-tissue factor/anti-factor VIIa, and tissue inhibitors of matrix metalloproteinases (TIMPs).¹⁴⁻¹⁸

Formation of new blood vessels can take place under physiologic or pathologic conditions. Physiologic, normal,

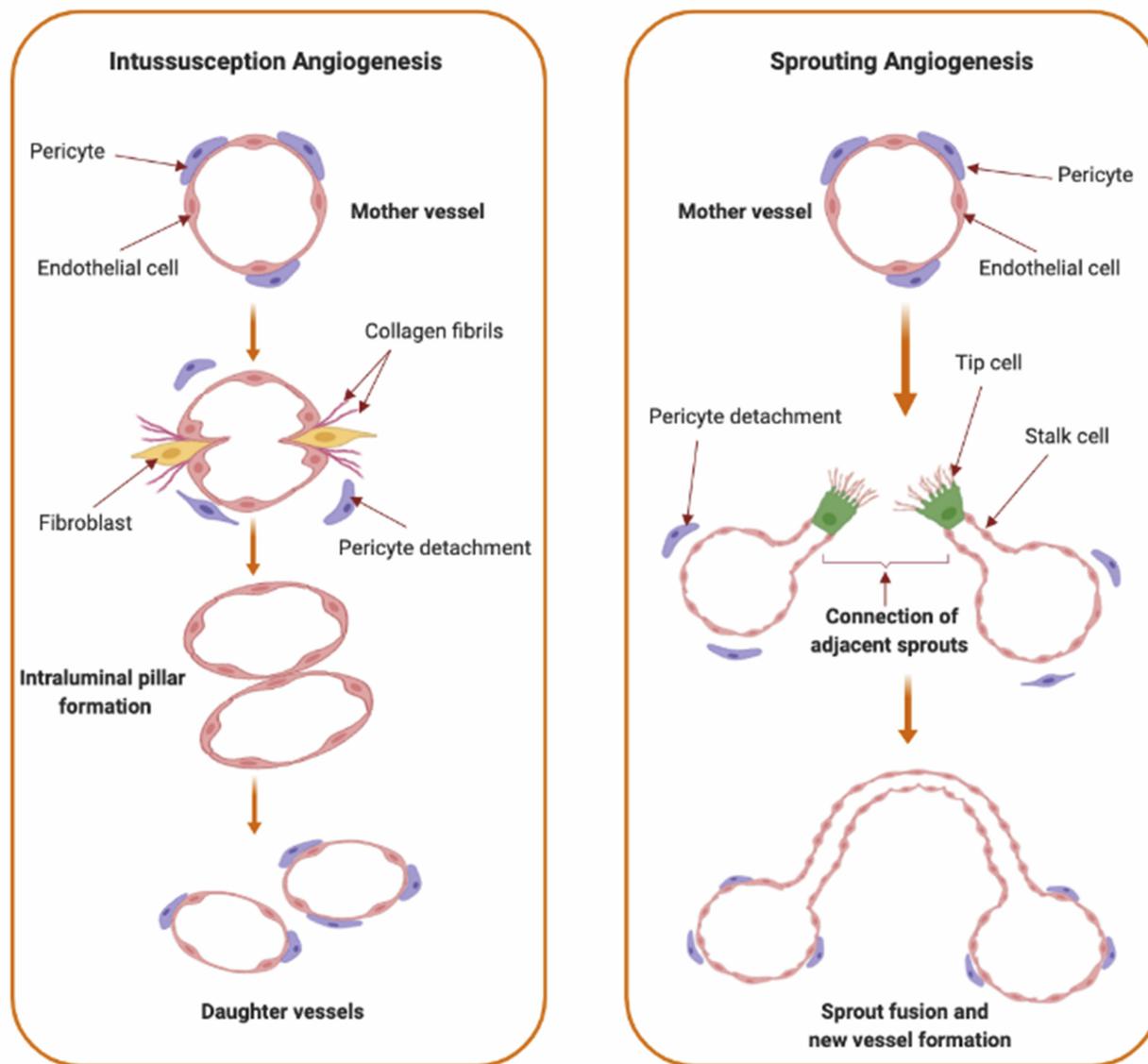


Figure 1 Types of angiogenesis. Types of angiogenic growth include intussusceptive (left panel) and sprouting angiogenesis (right panel). Intussusceptive angiogenesis, also known as splitting angiogenesis, represents the splitting of an existing blood vessel to form new blood vessels by a splitting process involving series of changes of interstitial tissues which invade the existing blood vessel in the presence of pericytes and fibroblasts which ends up with the core splitting by intraluminal pillars development of periendothelial cells and the formation of new smaller vessels or capillaries. Sprouting angiogenesis, as indicated from the name, involves the sprouting of endothelial cells from pre-existing blood vessel by the interruption of endothelial cells basement membrane, migration of endothelial cells along the projection's tip via stalk cells' formation into the surrounding connective tissue with further sprouting and new capillary network formation.

angiogenesis is regularly activated in the developing fetus and continues to form blood vessels of the adult tissues.¹⁹ Physiological angiogenesis is activated in response to different physiological stimuli, such as tissue hypoxia, inflammation, and/or injury, and plays a vital role in physiological processes such as wound-healing and embryonic development.⁷ However, under certain circumstances, the balance between angiogenesis stimulators and inhibitors is altered in favor of excessive angiogenic signaling, which ultimately drives pathological angiogenesis.^{9,20,21} Pathological angiogenesis involves the formation of vessels which are hyperpermeable to plasma and its proteins and possess heterogeneous functions with irregular sprouting and non-uniformed vessel distribution.^{22–26} Pathologic angiogenesis contributes to multiple disease conditions such as ocular neovascularization, cancer, and inflammatory disorders.¹³ Dysregulation of angiogenesis has been also shown to contribute to the pathogenesis of other disorders such as asthma, obesity, atherosclerosis, infectious diseases, heart and brain ischemia, pre-eclampsia, respiratory distress, hypertension, and osteoporosis.⁷ Targeting pathologic angiogenesis has widespread biomedical applications with the ultimate goals to control the rate of angiogenic growth and normalize vasculature in target tissues.

Targeting angiogenesis for therapeutic indications

The goal of therapeutic angiogenesis is to improve perfusion to ischemic tissues or organs by generating new blood vessels and stimulating neovascularization to restore tissue functions, or to inhibit abnormal angiogenesis in disorders like cancer.^{9,13} Although promising results were shown in experimental animal studies for pro-angiogenic factors, such results were not attained in clinical studies.^{27,28} Despite the fact that the challenges associated with clinical translation are specific for each therapeutic approach, many hurdles were attributed to drug delivery, such as failure to deliver the treatment to the designated target with the required amounts or for the required duration of time.^{29,30} Regranex gel, which contains becaplermin; a recombinant angiogenic growth factor, is the first and only US-FDA approved pro-angiogenesis product. Becaplermin attaches to PDGF receptors in the targeted wound and initiates angiogenesis in diabetic neuropathic ulcers that extend to subcutaneous tissues.^{31,32} Regranex gel showed a significant increase in

the incidence of complete ulcer healing in patients with full-thickness diabetic neurotrophic foot ulcers when compared to placebo.³³ Malignancies distant from the gel application site however have been reported in both clinical and post-marketing studies, in addition to an increase in the rate of death in patients using three tubes or more of Regranex gel.³⁴ Together, these adverse effects limited the clinical usefulness of this product and further warranted the need for more tolerable angiogenic modulators.

Several anti-angiogenic agents have been approved by the US-FDA for cancer therapy. Angiogenesis inhibitors include monoclonal antibodies that target specific pro-angiogenic growth factors and/or their receptors (bevacizumab and ramucirumab); tyrosine kinase inhibitors (TKIs) of growth factor receptors (sorafenib, sunitinib, axitinib, cabozantinib, pazopanib, regorafenib, and vandetanib); and inhibitors of mammalian target of rapamycin (mTOR) (temsirolimus and everolimus) (Figure 2).³² Despite the availability of anti-angiogenic drugs, many challenges limit their use in clinical practice. Among them is the development of more aggressive tumors once treatment is withdrawn.^{35,36} In addition, angiogenesis inhibitors have a wide range of serious adverse effects, such as bleeding, hypertension, leukopenia, and lymphopenia, which could further hinder their use in clinical settings. Angiogenesis inhibitors have been also shown to reduce delivery of chemotherapeutic drugs to target tumors and, in some cases, to affect the distribution and pharmacokinetic profiles of co-administered anti-cancer treatments.^{14,37–40} Furthermore, the acquired resistance or “escape” of cancer cells against these agents is considered one of the most important challenges that limit their efficacy.^{14,41–43} Moreover, angiogenesis inhibitors lack valid and standardized biomarkers for assessing their efficacy and/or tolerability, which further add to therapy limitations.^{14,41} Taken together, efforts to effectively target pathologic angiogenesis while overcoming current limitations to angiogenesis inhibitors are ongoing, especially in the area of cancer management. Targeting angiogenesis has been, without a doubt, impacted by technological advances in nanotechnology. The administration of angiogenesis modulators using nanoparticles, especially metal nanoparticles, has been increasingly utilized. This was driven by many advantages of nanoparticles; such as longer half-life, improved efficacy, and selective targeting of tumor-associated

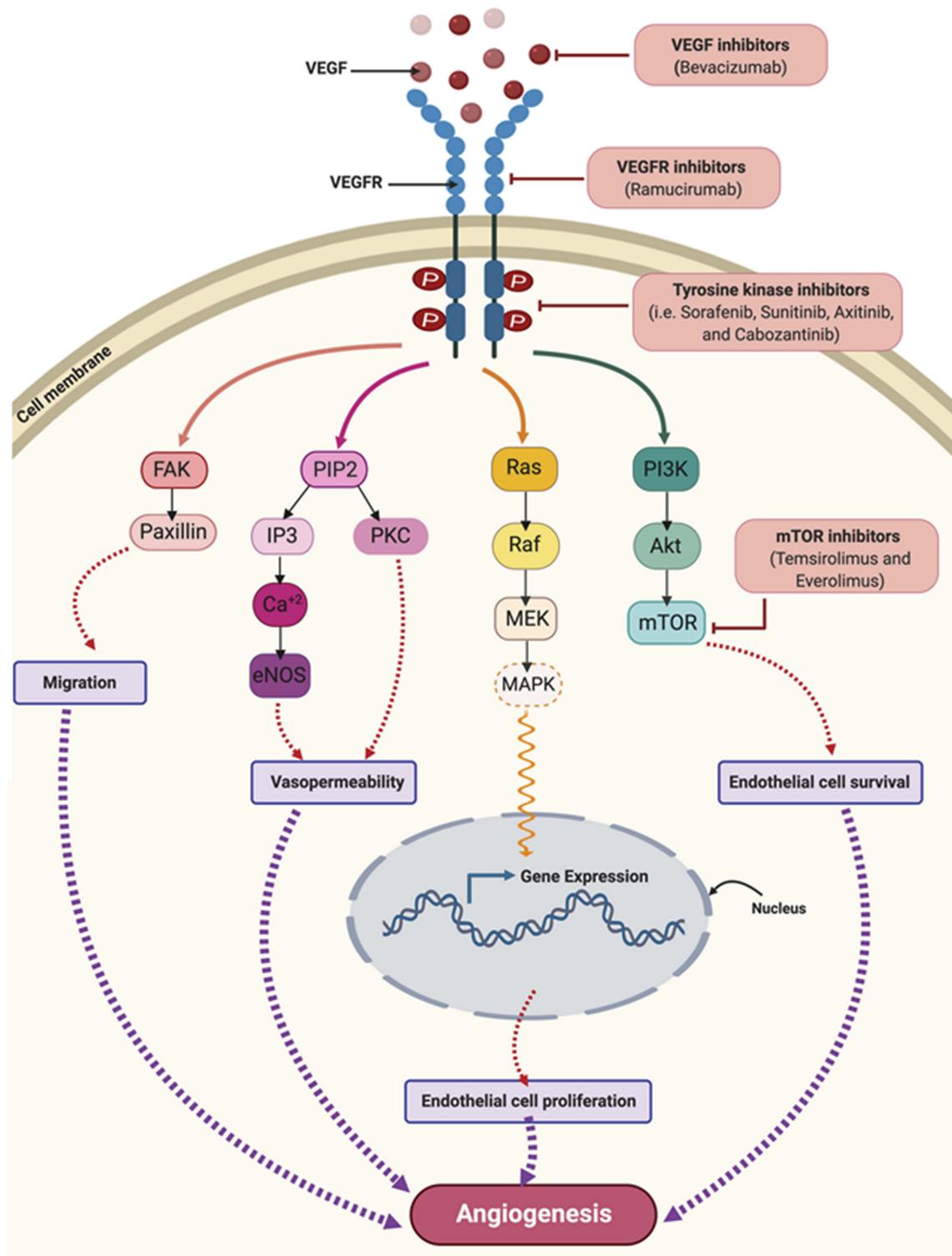


Figure 2 VEGF/VEGFR downstream signaling pathways and sites of actions of approved anti-angiogenic drugs in cancer therapy. Binding of VEGF to VEGFR leads to activation of multiple downstream cell signaling pathways including FAK/paxillin, PIP2, RAS/MAPK, and PI3K/Akt/mTOR-pathways. Monoclonal antibodies such as bevacizumab and ramucirumab bind to VEGF and VEGFR, respectively, preventing receptor activation and subsequent downstream signaling. Cabozantinib, pazopanib, regorafenib, sorafenib, sunitinib, and vandetanib are tyrosine kinase inhibitors which target the kinase domain of VEGFR. Temozolimus and everolimus are inhibitors of mTOR which is a downstream effector for the VEGF/VEGFR axis. Collectively, these drugs will abolish angiogenic signaling in endothelial cells. These anti-angiogenic effects occur via inhibiting endothelial cells migration, vasopermeability, proliferation and survival.

Abbreviations: Akt, protein kinase B; eNOS, endothelial nitric oxide synthase; FAK, focal adhesion kinase; IP3, inositol trisphosphate; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; PKC, protein kinase C; PIP2, phosphatidylinositol 4,5-bisphosphate; PI3K, phosphoinositide-3-kinase; RAF, rapidly accelerated fibrosarcoma; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

vasculature.^{39,44} Properties of nanoparticles that facilitate their utilization for biomedical applications include the enhanced permeability and retention effect (EPR), which allows the internalization of nanoparticles to tumor tissues, the ability to avoid the immune system, which prolongs the drug half-life and decreases its effective dose, and their high surface density, which results in selective targeting.³⁹

Among metal nanoparticles, gold nanoparticles (AuNPs) have captured recent attention for their potential use as angiogenic modulators. In the following subsections, the current evidence for utilizing AuNPs to modulate angiogenic growth along with the proposed molecular mechanisms and therapeutic applications for their effects are summarized. In addition, the physical characteristics and methods for the synthesis of AuNPs, as well as their uptake mechanisms, and toxicity are briefly outlined.

Gold nanoparticles (AuNPs)

AuNPs synthesis and properties

Gold nanoparticles (AuNPs) are composed of an inorganic core containing gold which is typically encircled by an organic monolayer.⁴⁵ A representative structural model of a single AuNP (Figure 3, upper panel), is called “monolayer protected cluster; MPC”. MPC usually consists of central inner gold atoms “core” that determine the crystallinity of the structure; gold atoms on the outer surface of AuNPs structure, which usually have a different geometry than the core atoms, that create the surface’s facets and edges that control the catalytic activity of the particle, and a protecting outer layer of organic ligands or surfactants.^{45,46} The latter structures stabilize AuNPs and provide them with surface functionality.^{45,46} Depending on the size ranges of AuNPs, they could form clusters, catalytic particles or plasmonic crystals with different nanostructures (Figure 3, lower panel).^{46,47} These include tetrahedrons,^{48,49} cubes,^{50–52}

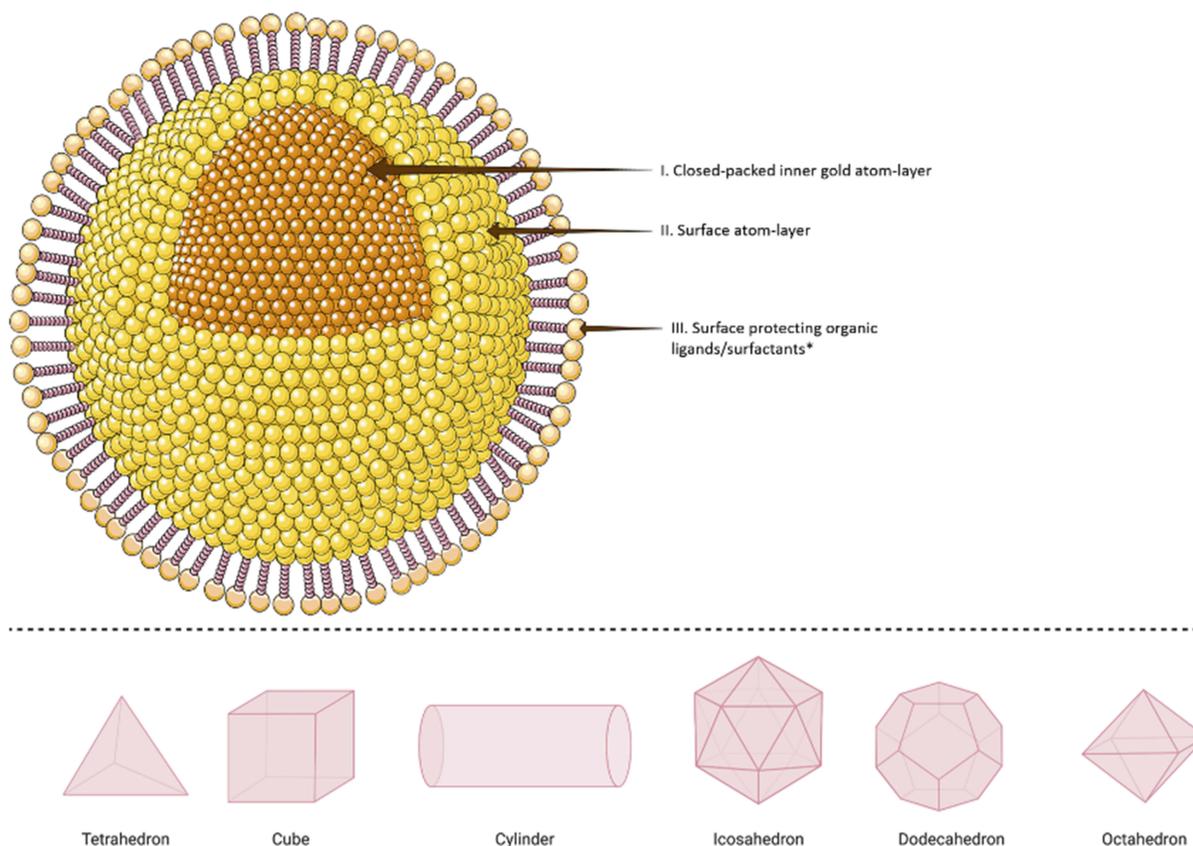


Figure 3 Schematic illustration of different shapes of a single crystalline gold nanoparticle (AuNP). Upper panel: In the simplest structure, AuNPs are commonly composed of central inner gold atoms with a closed-packed crystallographic face-centered cubic structure (domain I), surrounded by surface atoms on the outer surface of the gold atoms (domain II), which could be functionalized with an outmost layer of surface organic ligand or surfactants (domain III). The structure above is shown with a spherical shape, however, it could be also synthesized in various shapes as examples shown in 3D illustrations in the lower panel. *In the upper panel, the surface protecting ligands/surfactants were added to the posterior part of the spherical nanoparticle only for a clearer presentation of domains I and II.

cylinders,⁵³ icosahedron,⁴⁹ dodecahedrons,⁵⁴ octahedrons,⁴⁹ in addition to a variety of branched structures.^{55–58}

Depending on the composition of AuNPs, a wide variety of organic ligands could be introduced to functionalize the surface of AuNPs and enhance their properties. Such modifications in drug delivery are crucial for adequate drug loading, proper drug-releasing, cellular uptake, and targeting.⁵⁹ Surface functionalization is used to introduce numerous biomolecules to the surface of AuNPs, such as peptides, proteins, and oligonucleotides.^{60,61} For example, gold nanoclusters coated with a mixed monolayer of *n*-alkanethiol and tetraalkylammonium followed by DNA assembly on the surface of the AuNPs were used by McIntosh et al to inhibit transcription by T7 RNA polymerase in vitro.⁶² In addition, multifunctional AuNPs with polyethylene glycol (PEG), cell penetration and cell adhesion peptide, and small interfering RNA (siRNA) were tested for silencing *c-Myc* protooncogene, an important gene for cell cycle and homeostasis, both in vitro and in vivo.⁶³ Wu et al synthesized DNA gold nanoassembly composed of a gold core, a cationic peptide interlayer, and an outer layer of fluorophore-labeled hairpin DNA probes, which demonstrated highly efficient cellular delivery of DNA probes and overcome entrapment by endosomes.⁶⁴ Functionalization of AuNPs with a mixture of ammonium- and carboxylate- groups attached to the alkyl-thiol ligand terminals are also used to enhance cellular penetration and uptake.⁶⁵ Relevant functionalized AuNPs are discussed below while addressing the effects of AuNPs on angiogenesis.

Several methods, such as the Turkevich and Brust-Schiffrin methods, have been utilized for AuNP synthesis. The Turkevich method involves nucleation of gold ions through the reduction of gold salts by a reducing agent.⁶⁶ In this method, sodium citrate is added as a gold-reducing agent and capping agent to stabilize and prevent the aggregation of AuNPs (Figure 4A). Turkevich method results in monodispersed spherical AuNPs, in aqueous solution, that are 10–20 nm in diameter with a relatively narrow size distribution.⁴⁶ Turkevich method however is limited by the narrow size range of the synthesized AuNPs. It was later refined by Frens et al by varying the ratio of the reducing agent to the stabilizing agent to allow for the production of AuNPs with a wider size range.^{67,68}

The Brust-Schiffrin method was inspired by Faraday's two-phase system and results in large quantities of MPCs. The advantage of the Brust-Schiffrin method is that it

results in more stable AuNPs that are smaller in size and monodispersed in terms of shape and size. This method involves the transfer of gold ions from a gold salt aqueous solution into an organic solvent (e.g. toluene) using a phase-transfer agent such as tetraoctylammonium bromide (TOAB).⁶⁹ This step is followed by a thiol-mediated capping of the gold clusters usually by using a thiol-terminated long-chain alkane, which is followed by the addition of a reducing agent such as sodium borohydride in the presence of alkanethiols (Figure 4B). The thiol-mediated capping step prevents aggregation of the reduced gold atoms and results in the production of stable organic monolayers on the AuNPs.^{45,46,69–71} The synthesized AuNPs can be further modified by ligand place-exchange reactions with different biomolecules to add a variety of specific functionalities to AuNPs in a process called the Murray method (Figure 4C).^{45,46,72}

Turkevich and Brust-Schiffrin methods are usually used to produce spherical AuNPs. AuNPs can also be synthesized into different nanostructures, such as rods,⁷³ triangles,⁷⁴ wires,⁷³ cubes,⁵² tubes,⁷⁵ and stars⁷⁶ via alternate procedures. The most commonly used procedure for the synthesis of non-spherical AuNPs is the seed-mediated growth, known as Murphy method.^{77,78} The basic idea of this method is to produce seed particles by the reduction of gold salts using a small amount of a strong reducing agent (e.g. sodium borohydride). These seeds are then added to a metal salt solution in the presence of a weak reducing agent (e.g. ascorbic acid) and surfactant (e.g. cetyltrimethylammonium bromide, CTAB), which is used as surface protecting agent (Figure 5).^{46,77,78} The geometry of the targeted gold nanostructure can be determined by altering the concentration of seed particles, reducing agents and/or the surface protecting agents.^{70,77,78} Other methods for the synthesis of AuNPs include electrochemical-, photochemical-,^{79–81} sonochemical-,⁸² and microwave-assisted methods.⁸³ These methods use different reducing energy sources, such as electron-, ultraviolet-, ultrasonic-, and microwave-sources, respectively.⁴⁶

Despite the efficient synthesis of AuNPs using chemical methods, the generation of toxic byproducts along with the use of toxic materials and solvents are major concerns that could impact their large scale production and biological applications.^{70,84,85} Thus, synthetic procedures for AuNPs production have been devised using biological components that lack toxicity. Green biosynthesis of AuNPs have many advantages when compared to chemical synthesis; such as reduced

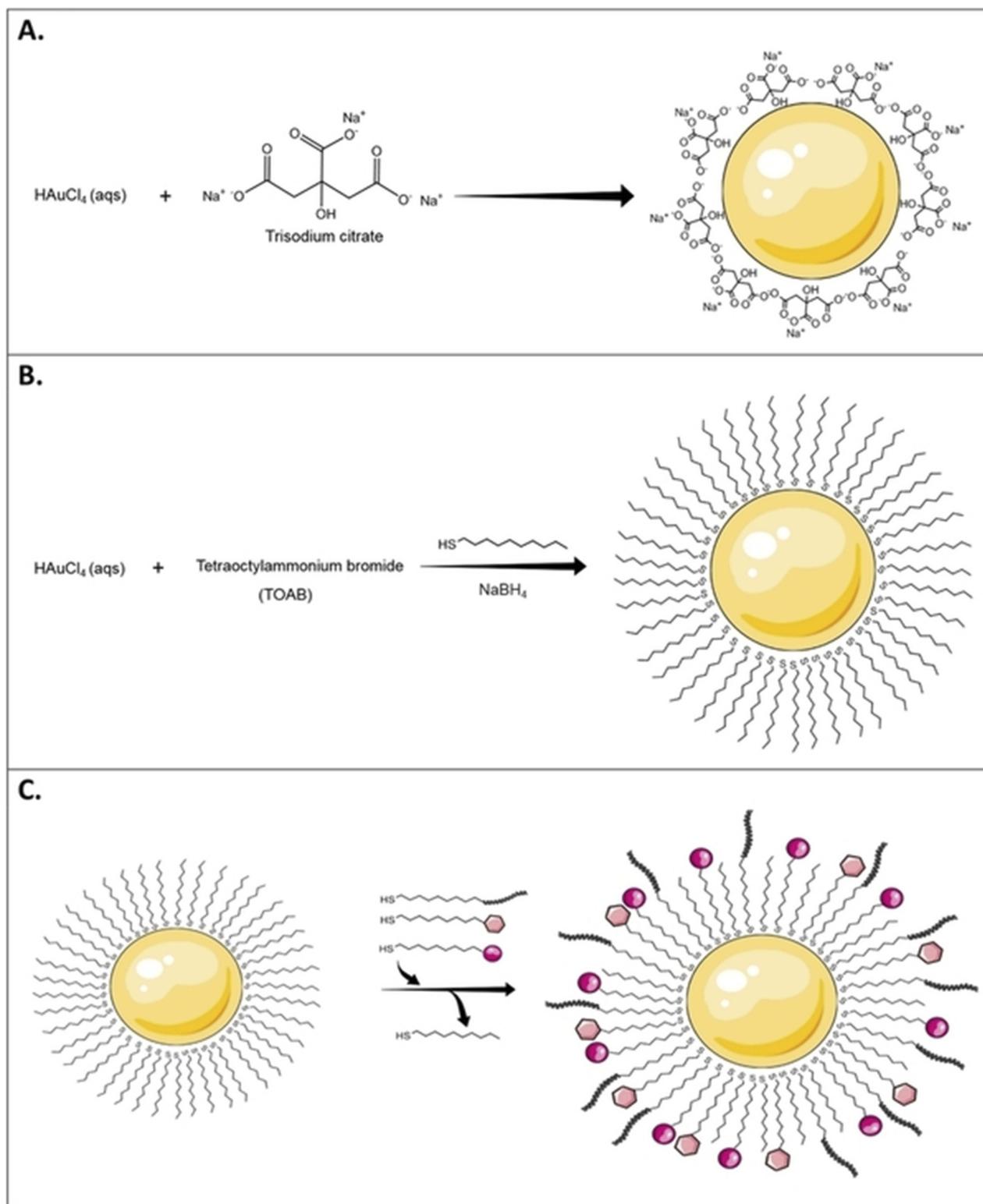


Figure 4 A schematic illustration of different AuNPs synthesis methods. **(A)** Turkevich method. This method uses sodium citrate as a gold-reducing agent and capping agent, **(B)** Brust-Schiffrin method includes the use of phase transfer agent (TOAB) and thiol-mediated capping of AuNPs, and **(C)** Murray method or place-exchange method, represented in organo-thiol (-SH) system, which can be used with different ligands and biomolecules.

toxicity, lower cost, and safety to the environment.⁷⁰ Several biological resources were utilized in the synthesis of AuNPs

such as plants or plant extracts,^{86–88} microorganisms,^{89–92} and algae.^{93,94} In addition, biomolecules have been used including

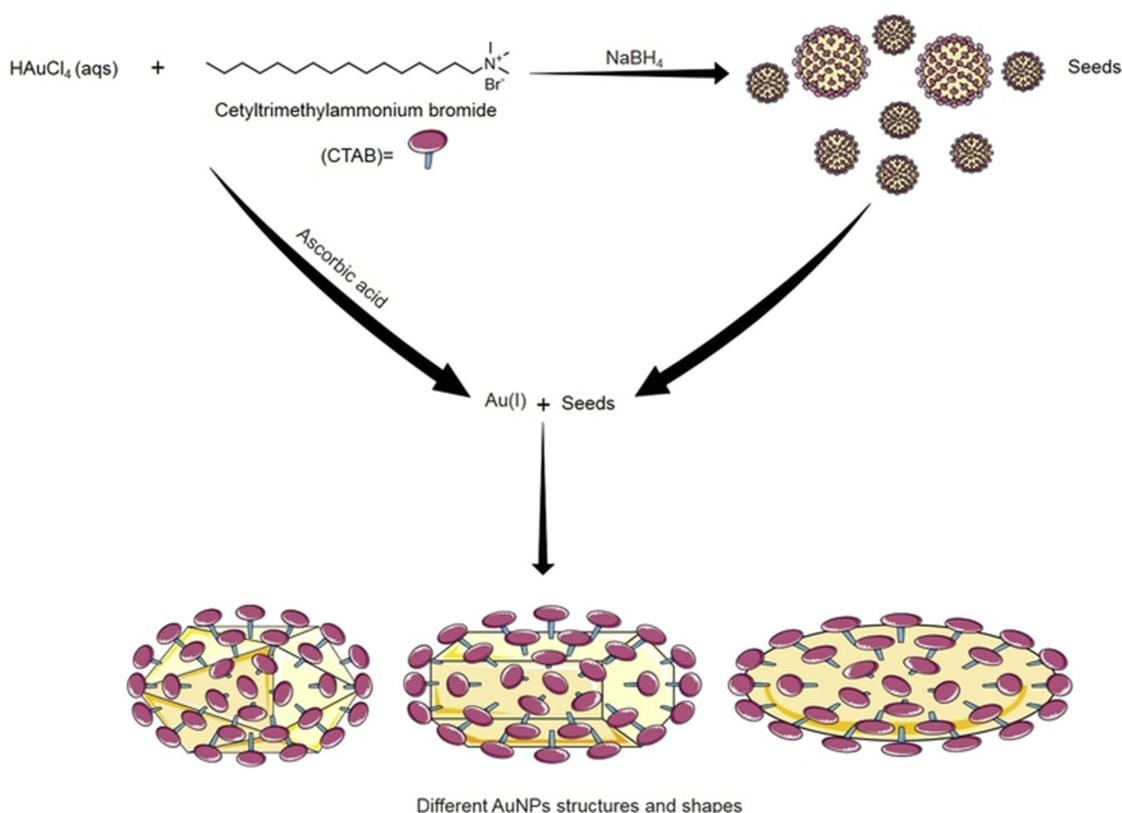


Figure 5 The seed-mediated growth synthesis method of different shapes of AuNPs. In Seed-mediated growth method, seed particles are produced by the reduction of gold salts using a small amount of a strong reducing agent (e.g. Sodium borohydride; NaBH_4). Then these seeds are added to a metal salt solution in the presence of weak reducing agent (e.g. ascorbic acid) and surfactant (e.g. cetyltrimethylammonium bromide; CTAB). Changing the concentration of seed particles, reducing agents and/or the surface-protecting agents control the geometry of the resulted gold nanostructure.

amino acids,^{95–97} enzymes,⁹⁸ lipids,^{99–101} nucleic acids,^{102,103} fatty acids,¹⁰⁴ carbohydrates,¹⁰⁵ and polysaccharides.^{106,107}

Cellular uptake and internalization of AuNPs

Several internalization mechanisms were suggested and studied for nanoparticles. In phagocytic cells, phagocytosis is a common mechanism for internalization of large clusters or aggregates of nanoparticles. Alternatively, nanoparticles are internalized by pinocytosis or passive uptake through the cell membrane in non-phagocytic cells.^{108,109} Depending on different nanoparticles properties, functionalization, and surface modifications; they could be internalized by either receptor-mediated endocytosis, or non-specific receptor-independent endocytosis.^{108–110}

Cellular uptake and internalization pathways of AuNPs (Figure 6) are affected by many factors such as particle size, shape, surface charge, and functionalization.^{108,109} The effect of the size of AuNPs on their cellular internalization was widely studied. Chithrani et al showed that

50 nm AuNPs had the highest uptake by HeLa cells among a range of tested sizes; 10–100 nm.¹¹¹ Similarly, Ko et al demonstrated that the uptake of 50 nm AuNPs by human adipose-derived stem cells was more than other examined sizes; 15–100 nm.¹¹² However, other studies showed that cellular uptake of smaller gold nanoclusters (1–2 nm) was greater than larger gold nanoclusters (12 nm).^{113,114}

Surface coating and functionalization play a vital role in determining cellular uptake mechanism. Saha et al showed that the uptake of cationic AuNPs involves different endocytic pathways (caveolin- and dynamin-dependent pathways) and specific cell surface receptors, in HeLa cells and MCF10A cells.¹¹⁵ On the other hand, coated AuNPs with PEG, polyvinyl alcohol (PVA) or a mixture of both demonstrated different uptake behaviors by monocyte-derived dendritic cells (MDDCs), as (PEG+PVA)-AuNPs and PVA-AuNPs showed higher uptake, compared to PEG-AuNPs.¹¹⁶

In regards to AuNPs shape, Xie et al showed that AuNPs with triangular shape resulted in the highest uptake by RAW 264.7 macrophages, compared to rods and

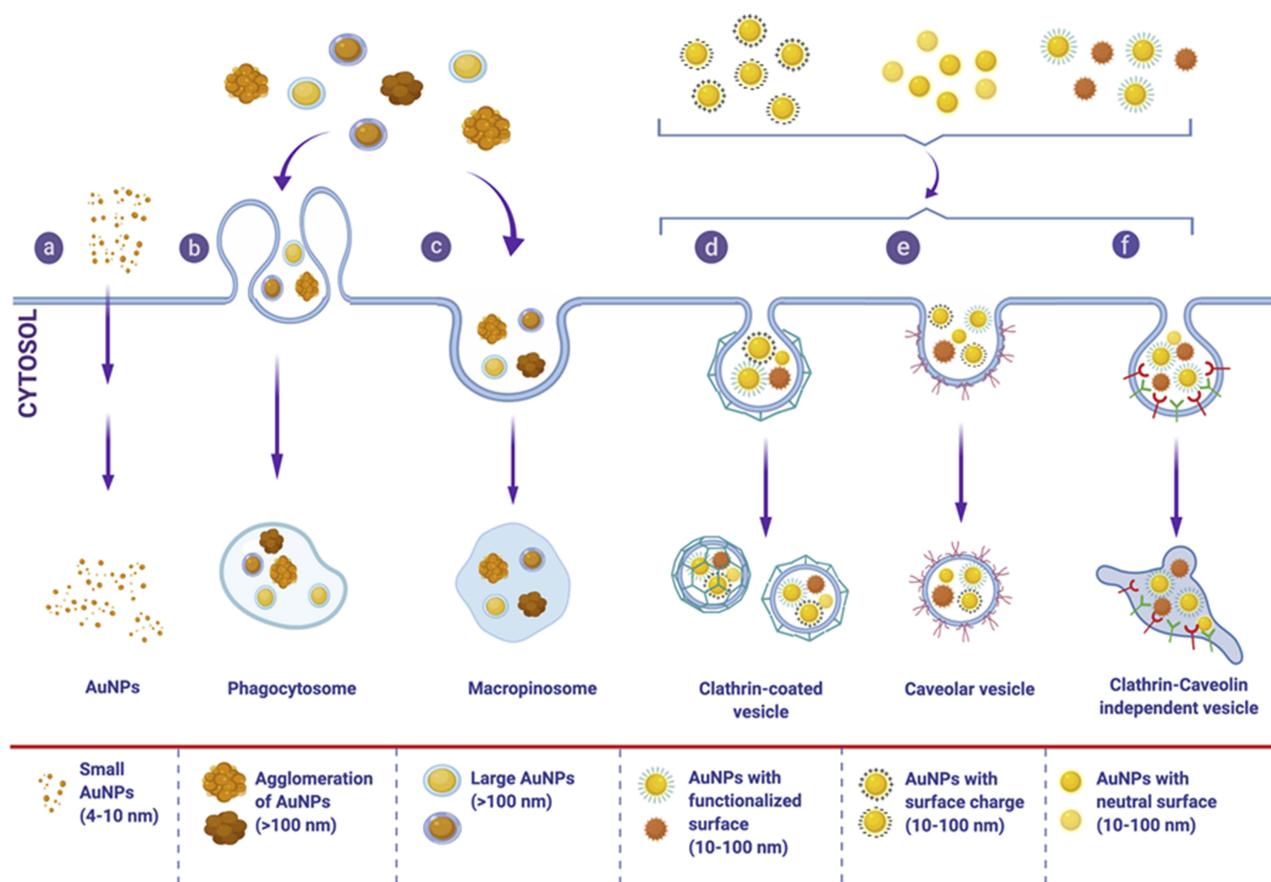


Figure 6 Internalization and cellular uptake pathways of AuNPs. Different properties of AuNPs, such as particle size, shape, surface charge, and functionalization affect internalization and cellular uptake mechanisms in different cells. These pathways include: (a) passive uptake for small AuNPs, (b) phagocytosis and (c) micropinocytosis for large size nanoparticle or aggregates of AuNPs, different pinocytosis pathways; (d) clathrin-dependent endocytosis, (e) caveolin-dependent endocytosis, and (f) clathrin/caveolin-independent endocytosis which are usually the uptake pathways for AuNPs with functionalized-, charged- or neutral-surfaces. Spherical AuNPs are used for presentation purposes only, different shapes of AuNPs are internalized by a variety of cells and affect the internalization pathways as well.

stars.¹¹⁷ In addition, each shape has different endocytosis pathway; triangles are internalized via clathrin-mediated endocytosis and dynamin-dependent pathway, rods through both clathrin- and caveolin-mediated endocytosis, and stars through clathrin-mediated process.¹¹⁷ Overall, the heterogeneity of AuNPs' properties and synthesis methods determine their cellular internalization and their intracellular fate.

AuNPs and angiogenesis

The anti-angiogenic effects of AuNPs have been demonstrated in multiple studies. AuNPs reduced viability, migration, and tube formation of human umbilical vein endothelial cells (HUVECs).¹¹⁸⁻¹²⁶ The anti-angiogenic effects of AuNPs on HUVECs were found to be dose-dependent.^{118,119,122} AuNPs were also shown to inhibit VEGF-A-induced migration of human retinal microvascular endothelial cells (HRMECs) in vitro.¹²⁷ The anti-angiogenic activity of AuNPs has been

further demonstrated utilizing ex vivo models such as the chick chorioallantoic membrane model (CAM).^{128,129} In the CAM model, AuNPs inhibited formation of new blood vessels, reduced total tubule complex, total vessel length, and number of vessel junctions.^{124,128}

Administration of AuNPs has also been shown to result in anti-angiogenic activity in multiple models in vivo. AuNPs reduced vascular density and permeability while maintained vessel integrity in different tumor models. AuNPs treatment normalized tumor vasculature in nude mice inoculated with SW620 human colorectal cancer.¹³⁰ AuNPs reduced vascular density as indicated by staining tumor tissues with CD31 vessel marker. In addition, AuNPs improved vascular stability by increasing pericyte coverage as determined by detection of α -smooth muscle actin on tumor vasculature within 6-9 days of treatment.¹³⁰ In an animal model of melanoma, the anti-angiogenic activity of AuNPs was demonstrated in terms of reduced vascular

density, reduced permeability, and improved vascular morphology and perfusion.¹³¹ Normalization of tumor vasculature was associated with reduced lung metastasis in AuNPs-treated animals when compared to vehicle-treated controls.¹³¹ In an animal model of liver cancer, the anti-angiogenic activity of AuNPs revealed a remarkable decrease in vascular density in treated animals as indicated by staining for the marker CD34.¹²¹ Furthermore, AuNPs reduced vessel permeability in a mouse ovarian tumor model as indicated by reduced fluid accumulation in the peritoneal cavity of nanoparticle-treated animals.¹³² A reduction in angiogenesis was also observed in a nude mouse ear model treated with AuNPs.¹³²

Molecular mechanisms associated with anti-angiogenic effects of AuNPs

Multiple molecular pathways have been shown to contribute to the anti-angiogenic effects of AuNPs (Figure 7). The VEGF-A/VEGFR pathway has been a major molecular target mediating the anti-angiogenic activity of AuNPs. Accumulating evidence revealed that treatment with AuNPs suppressed the activity of VEGF-A/VEGFR2 axis and its downstream signaling in different endothelial cells both in vitro and in vivo. AuNPs were shown to decrease expression of VEGF-A and suppress VEGFR2 phosphorylation in HUVECs.^{123,125,126,133–135} Inhibition of the VEGF-A/VEGFR2 axis led to subsequent inhibition of AKT and ERK1/2 phosphorylation in endothelial cells.^{121,124,134} In addition, AuNPs were shown to suppress intracellular calcium release and reduce RhoA activity mediated by VEGF-165 in HUVECs.^{121,133} AuNPs have been shown to downregulate the levels of pro-angiogenic factors, Ang-1 and Ang-2, in tumor tissues.¹³⁵ In addition, Vimalraj et al indicated that AuNPs exerted anti-angiogenic effects in CAM model by downregulating Ang-1/Tie2R pathway.¹²⁹

Animal studies showed that AuNPs suppressed inflammatory cytokines and release of MMPs. AuNPs depleted MMP-2 in H-357-PEMT cells in a p53- and p21-dependent manner.¹³⁵ They altered the level of inflammatory cytokines IL-6, IL-1 β , TNF- α , and metastasis-associated markers (CD-44, CD-133) in H-357-PEMT cells and conditioned media-treated HUVECs in a p53/p21-dependent manner.¹³⁵ AuNPs decrease the production of IL-12, IL-6, MMP-1, and TNF- α from dermal fibroblasts suppressing angiogenesis.¹³⁶ AuNPs reduced epithelial-to-mesenchymal transition (EMT) as indicated by upregulation of the epithelial marker E-cadherin and downregulation of the mesenchymal protein, vimentin.¹³¹

AuNPs also inhibited angiogenesis by reducing MMP-2 expression and increasing the levels of the junctional adaptor protein ZO-1 in endothelial cells.¹³¹ AuNPs further inhibited basic FGF-2-induced angiogenesis.^{126,136} Recently, Shen et al showed that AuNPs inhibited retinal neovascularization through induction of autophagy, which was confirmed by upregulation and detection of autophagic markers LC3-II, ATG5, Beclin1, and p62.¹²² The molecular pathways associated with the anti-angiogenic activity of AuNPs are illustrated in Figure 7.

Biomedical applications of AuNPs

The unique physical and chemical properties of AuNPs make them the perfect choice for a variety of biomedical applications. AuNPs represent an appealing choice as angiogenic modulators due to their compatibility, along with their ease of synthesis and characterization owing to the presence of surface plasmon resonance band.^{132,137–140} AuNPs can bind to amine- and/or thiol-groups in proteins, which represent unique ligands for AuNPs.^{23,133} In addition, AuNPs can be produced in a wide range of particle size and shapes to meet the indicated application. The vascular activity of AuNPs has been particularly important in mediating the medical applications for these nanoparticles. The medical applications of AuNPs that are associated with modifying the angiogenic process and vasculature are summarized below.

AuNPs as therapeutic agents in pathologic angiogenesis

Administration of AuNPs has been shown to block pathological angiogenesis in the retina, a leading cause of blindness.^{127,134} Intravitreal administration of AuNPs significantly inhibited retinal neovascularization in a mouse model of oxygen-induced retinopathy of prematurity when compared to control animals.¹³⁴ Histological examination showed no retinal changes in terms of thickness, inflammation, or cytotoxic effects to retinal cells in AuNPs treated-animals compared to vehicle-treated groups.¹³⁴ Reva et al examined the effect of subcutaneous injections of AuNPs on dermal structures in male CBA mice.¹²⁵ Injected nanoparticles were phagocytosed by macrophages and the anti-angiogenic activity of AuNPs was mediated by the toxic activity of macrophages loaded with nanoparticles. Cytotoxic activity of AuNPs on vascular endothelium in subcutaneous tissues was mediated by the deactivation of macrophages that produce VEGF-A, or through direct death

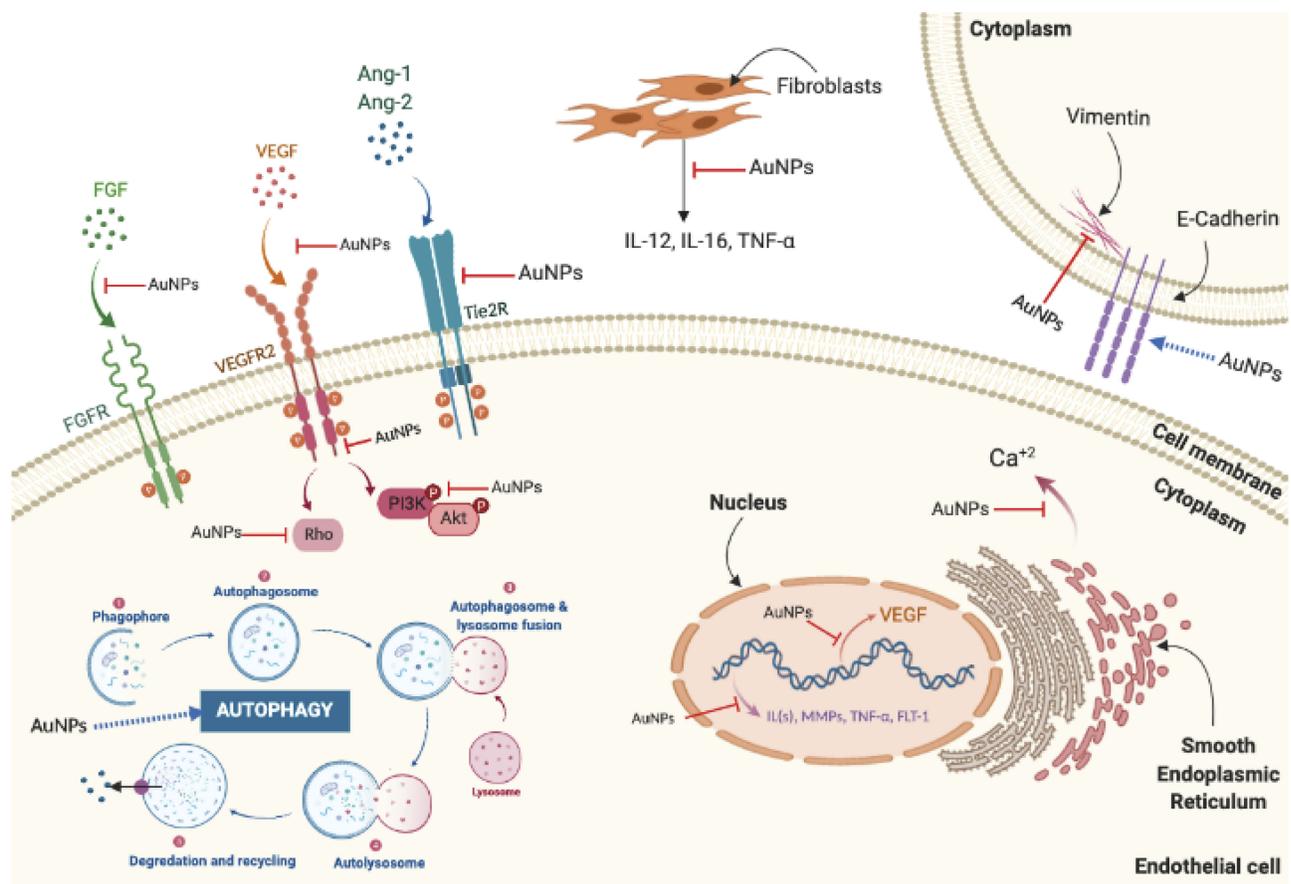


Figure 7 Molecular pathways affected by the anti-angiogenic effects of AuNPs. Anti-angiogenic effects of AuNPs are mediated by suppressing activation of VEGFR2, Tie2R, FGFR, and their downstream signaling pathways. AuNPs suppress intracellular calcium release mediated by VEGF-I65. AuNPs upregulate E-cadherin and downregulate vimentin reducing epithelial-to-mesenchymal transition (EMT). AuNPs reduce ILs, MMPs, and TNF- α expression and inhibit neovascularization via induction of autophagy. **Abbreviations:** Ang-1, angiopoietin 1; Ang-2, angiopoietin 2; Ca²⁺, calcium; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; ILs, interleukins; MMPs, matrix metalloproteases; Tie2R, angiopoietin receptor; TNF- α , tumor necrosis factor- α ; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

of the endothelium as a result of the migration of macrophages through the vascular wall.¹²⁵ AuNPs treatment normalized tumor vasculature in a xenograft nude mice model of human colorectal carcinoma (CRC).¹³⁰ Treatment reduced expression of VEGFR2 and HIF-1 α , which was accompanied by reduced vessel hyperpermeability and hypoxia, respectively. In addition plasma level of anterior gradient 2 (AGR2), a biomarker associated with cancer progression and angiogenesis, was reduced as a result of AuNPs treatment. Interestingly, effects on tumor vasculature have been found to abolish by day 14 of AuNPs treatment suggesting a time frame for the anti-angiogenic effects of AuNPs.¹³⁰

Other studies have demonstrated that AuNPs could enhance angiogenesis in animal models. The application of AuNPs in photo-bio-modulation therapy (PBMT) accelerated cutaneous wound healing in Sprague Dawley rats. Histological results indicated that AuNPs and PBMT is

effective in stimulating angiogenesis and triggering inflammatory response at an early stage due to enhanced epithelialization, collagen deposition, and fast vascularization.¹⁴¹ In addition, AuNPs increased the expression of CD31 endothelial marker and enhanced angiogenesis in an orthotopic co-implantation model of pancreatic cancer.¹⁴² Roma-Rodriguez et al demonstrated modulation of angiogenesis using AuNP-peptide conjugates in CAM assay.¹⁴³ Specific peptide conjugates on the surface of AuNPs were observed to promote or inhibit angiogenesis in CAM assay by interacting with endothelial cell angiogenic receptors or by altering the balance between pro- and anti-angiogenic factors.¹⁴³

AuNPs for targeted drug delivery

Targeted AuNPs have emerged as potential delivery systems due to the ease by which they can be modified and the possibility of loading them with drug therapies. Arginine-glycine-aspartic acid peptide (RGD) has been widely used

as a targeting ligand because of its selective binding to the $\alpha\beta3$ and $\alpha\beta5$ integrins.¹⁴⁴ These integrins are overexpressed in endothelial cells during tumor angiogenesis.¹⁴⁴ Multiple studies used RGD-modified AuNPs for detection of cancer angiogenesis and for tumor-targeted delivery in vivo. Peptides based on the cyclic RGD sequence have been designed to antagonize the function of $\alpha\beta3$ integrin, thereby inhibiting angiogenesis in $\alpha\beta3$ -integrin-positive C6 gliomas.¹⁴⁵ In another study, radiolabeled ¹⁷⁷Lu-AuNP-RGD were found to deliver the highest tumor radiation absorbed dose and to induce less tumor progression and metabolic activity than ¹⁷⁷Lu-AuNP or ¹⁷⁷Lu-RGD radiopharmaceuticals in glioma cells of athymic mice. ¹⁷⁷Lu-AuNP-RGD decreased tumor vascularity and VEGF-A gene expression.¹⁴⁵

Li et al investigated the anti-tumor effects for the co-delivery of the anti-angiogenesis drug captopril with siRNA loaded into AuNPs in an animal model of breast cancer.¹⁴⁶ The complexes started to accumulate at the tumor site by passive targeting through enhanced permeability and retention effect within 1 h of administration. In vivo investigation on nude mice bearing MDA-MB-435 tumor xenografts revealed that captopril/siRNA/AuNP complexes possessed satisfying tumor homing ability and strong anti-tumor activity, which was further associated with downregulating VEGF-A mRNA and protein levels in tumor tissue.¹⁴⁶ A hybrid-nanoparticle using quinacrine and gold (QAuNP) induced anti-angiogenic and anti-metastatic effect on oral squamous cell carcinoma (OSCC). QAuNP significantly suppressed angiogenesis and induced tumor regression in xenograft mice model of OSCC in vivo.¹³⁵ Saber et al prepared and tested the effect of c(RGDfK) labeled chitosan-capped AuNPs [cRGD(CS-Au)NPs] as a carrier for selective intracellular delivery of sunitinib malate (STB) to the tumor vasculature. cRGD(CS-STB-Au)NPs were more toxic to cells than the free STB with the same drug dose indicating that the prepared nanoparticles improved delivery and uptake by cells.¹²⁰

Recently, recombinant human endostatin conjugated AuNPs (rhES-AuNPs) have shown significant activity in inhibiting AGR2-induced angiogenesis in animal model of metastatic colorectal cancer (mCRC).¹¹⁸ AuNPs normalized vasculature by promoting vessel stability, indicated by increasing pericyte expression, and reducing VEGFR2 in mCRC xenografts. There was however no significant difference in vascularity between the control group and the rhES-AuNPs-treated group as determined by the

endothelial marker CD31.¹¹⁸ In agreement, Li et al reported on the targeted delivery of rhES using AuNPs as a drug delivery system in mice bearing H22 xenografted tumors.¹⁴⁷ rhES-AuNPs-PEG improved pericyte coverage and enhanced maturity and stability of tumor vessels.¹⁴⁷ AuNPs have been successfully used for the dual delivery of the chemotherapeutic drug doxorubicin and the anti-angiogenic drug sorafenib in tumor-bearing mice.¹⁴⁸ Intravenous administration of AuNPs carrying therapeutic drugs was found to accumulate at the tumor site.¹⁴⁸ Balakrishnan et al demonstrated a powerful anti-tumor activity for the bioflavonoid quercetin formulated into AuNPs-based drug delivery system in 7,12-dimethylbenz (a)anthracene induced mammary carcinoma in Sprague-Dawley rats.¹¹⁹ The quercetin-AuNPs delivery system suppressed mammary tumor growth in vivo as a result of tumor EMT suppression and angiogenesis.¹¹⁹ The conjugation of AuNPs and snake venom protein toxin NKCT1 showed anti-cancer effects, both in vivo and in vitro, in Ehrlich ascites carcinoma (EAC) cells and EAC induced mice. NKCT1-AuNP reduced proliferation and induced apoptosis, which resulted in reduced volume and weight of EAC tumors in male albino mice. These effects were mediated by decreased expression of VEGF-A, IL-10, and Bcl2, along with upregulation of Bax and caspase 3/9 in NKCT1-AuNP-treated animals.¹⁴⁹

AuNPs have also been modified to enhance angiogenesis in animal models. Kim et al examined the therapeutic effect of phytochemically stabilized AuNPs (pAuNPs) coated on a hydrocolloid membrane (HCM) for curing cutaneous wounds in Sprague Dawley rats.¹⁵⁰ Topical application of pAuNP-HCM enhanced skin regeneration and wound closure in injured animals. These effects were achieved through increasing collagen expression, enhanced expression of VEGF-A and angiopoietins, decreased MMP-1 and TGF- β 1 expression, along with enhanced antioxidant effect in AuNPs-treated animals.¹⁵⁰ Conjugating AuNPs with VEGF-A for the efficient transdermal delivery of VEGF-A in the treatment of wound injuries in BALB/c mice was shown to improve vascular density and increase the number of subcutaneous vessels in the experimental group that was treated with VEGF-A-AuNP when compared to control group.¹⁵¹ Topical application of anti-oxidant agents with AuNPs enhanced wound healing of diabetic mouse skin, an effect which was mediated by reduced expression of the receptor for advanced glycation end-products (RAGE) and increased VEGF-A levels.¹⁵²

Other examples of AuNPs conjugates were reported that combined the advantages of AuNPs with added components, such as hybrids of polyethylenimine/RNA-based AuNPs that combined the self-assembly and anti-VEGF-A characteristics of nucleic acid that inhibit tumor angiogenesis at its initial progression, and the capability of AuNPs to generate heat which resulted in photothermal treatment of cancer at its later stages.¹⁵³ Likewise, Nethi et al synthesized bio-conjugates of AuNPs using *Hamelia patens* (HP) leaf extract as a reducing and stabilizing agent,¹⁵⁴ which is also used as a wound-healing agent.¹⁵⁵ These AuNPs-HP conjugates introduced an eco-friendly green and cost-effective synthesis of AuNPs in addition to showing pro-angiogenic effects in both in vitro and ex vivo models.¹⁵⁴ Moreover, peptide conjugates with AuNPs were assessed by Wang et al. A multivalent ligand of a peptide sequence (REDV)-modified alginate (ALG) conjugated with AuNPs (cREDV-ALG) was prepared to promote angiogenesis.¹⁵⁶ REDV-peptide sequence was chosen as an adhesion peptide that adheres to $\alpha_4 \beta_1$ integrin, which is expressed on the membrane of endothelial cells, to enhance the binding between endothelial cells and ligand modified scaffolds in tissue engineering.^{156,157} cREDV-ALG showed a selective adhesion to HUVECs and promoted their proliferation.^{156,157} In addition, cREDV-ALG stimulated new vessel formation and increased blood vessel density in animal models.^{156,157} Altogether, findings from these studies demonstrated the potential use of AuNPs as a promising modality for targeted drug and gene delivery, both systemically and topically.

AuNPs for medical imaging

The application of AuNPs in radiology is aimed at overcoming some of the limitations of traditional imaging techniques, such as shallow penetration along with reduced sensitivity and specificity.¹⁵⁸ Tumor tissues have shown higher retention of AuNPs compared to traditional contrast agents thus improving tumor detection as the clearance of AuNPs from the blood is slower compared to iodine agents, allowing for longer imaging times.¹³⁹ Clark et al reported on the potential use of AuNPs as contrast agents for computed tomography (CT) using a primary mouse model of soft-tissue sarcoma.¹⁵⁹ The protocol identified changes and improved limits of detectability for tumor vasculature in vivo.¹⁵⁹ Furthermore, AuNPs were applied as signal amplifiers in multispectral optoacoustic tomography (MSOT) to visualize gastrointestinal cancer in an animal model.¹⁶⁰

Results showed that PEGylated AuNPs have the capacity to penetrate tumors and provide high-resolution signal amplification for optoacoustic imaging.¹⁶⁰

AuNPs have been modified with radioactive material for in vivo imaging. Radioactive iodine-labeled, cyclic RGD-PEGylated AuNP probes were designed to test for selective targeting on tumors in experimental models.¹⁶¹ Intravenously injected probes revealed rapid and effective accumulation of the probes within 10 min after injection, which was increased with time. Analysis of tumor tissue revealed uptake of ¹²⁵I-cRP-AuNPs via integrin $\alpha\beta_3$ -receptor specific endocytosis.¹⁶¹ Similarly, Morales-Avila et al showed effective accumulation of technetium-99m-labeled AuNPs conjugated to RGD [^{99m}Tc-AuNP-RGD] into tumor tissue after both intravenous and intraperitoneal injections.¹⁶² Intravenous administration of ^{99m}Tc-AuNP-RGD however resulted in a faster tumor uptake when compared to the intraperitoneal mode of administration.¹⁶² Pan et al reported that photoacoustic tomography combined with $\alpha\beta_3$ -gold nanobeacons demonstrated high resolution of in vivo angiogenesis imaging.¹⁶³ Using a mouse Matrigel-plug model of angiogenesis, $\alpha\beta_3$ -gold nanobeacons enhanced contrast and detection of both existing and sprouting neovessels. Interestingly, the nanoparticles showed specific homing towards immature neovessels but not mature ones.¹⁶³ AuNPs have been also examined for magnetic resonance imaging (MRI) indications. Zhang et al investigated thiol-PEG-carboxyl-stabilized Fe₂O₃/AuNPs targeted to CD105 for the detection of tumor angiogenesis in breast cancer xenografts. Tumor angiogenesis was detected using MRI at different time points after intravenous administration of nanoparticles to tumor-bearing mice.¹⁶⁴

AuNPs for photothermal therapy

AuNPs-mediated photothermal therapy has been thoroughly examined for cancer therapy in animal models and in clinical studies.^{165–167} Laser irradiation of AuNPs that were accumulated in tumor tissues resulted in the destruction of the tumor tissue while avoiding damage to healthy tissues as a result of selective light absorption and hyperthermia that was generated at the tumor site.^{165,167,168}

Gum Arabic-AuNPs associated with laser exposure produced apoptotic, anti-inflammatory, lipid peroxidation, and anti-neovascular effects in an animal model of lung cancer.¹⁶⁹ The apoptotic effects were mediated by elevations of cytochrome-c, death receptor 5, and caspase-3, while the anti-angiogenic activity was associated with a

reduced VEGF-A expression in tumor tissue.¹⁶⁹ An $\alpha v\beta 3$ integrin-targeted multi-modal nanoprobe, dendrimer-RGD (Den-RGD), was designed and intravenously injected into tumor-bearing mice xenografts at 24 h after photothermal therapy.¹⁷⁰ Authors showed that AuNPs modified with carboxylated bovine serum albumin had both anti-tumor and anti-angiogenesis effects under near-infrared laser irradiation in human tumor-bearing mice xenografts, suggesting the efficacy of AuNPs for photothermal therapy *in vivo*.¹⁷⁰ Interestingly, Pedrosa et al demonstrated the possibility of producing local anti-angiogenic effects through specific laser irradiation of target blood vessels that were treated with AuNPs conjugated to anti-angiogenic peptides *in vivo*. In this study, a green laser coupled to AuNPs generated high localized temperatures that were sufficient to precisely cauterize blood vessels to be applied in cancer therapy.¹⁷¹ Photoacoustic imaging conducted by irradiation with RGD peptides conjugated plasmonic gold nanostars with a near-infrared pulse laser demonstrated deep imaging and homogenous resolution for early diagnosis of tumor angiogenesis.¹⁷² Tumor vessel specific ultrafine AuNPs, which were anchored with anti-RhoJ antibody and conjugated to iodine (AuNP-I), were applied as targeted radiosensitizers.¹⁷³ Patient-derived xenograft tumor model was constructed through orthotopic transplantation of human breast tumor tissue into mice. AuNPs-I showed selective binding to tumor vessels, rather than normal vessel. Targeted photothermal treatment remarkably reduced the number of tumor blood vessels when compared to animal groups that were treated with radiation alone or with the angiogenesis inhibitor bevacizumab. The anti-angiogenic effects of targeted therapy were sustained for 30 days.¹⁷³ PEGylated gold nano-semicubes (PEG-GNSCs) also produced anti-angiogenic effects in an animal model of skin cancer.¹⁷⁴ Photothermal treatment with laser-stimulated PEG-GNSCs significantly reduced VEGF-A concentrations in tumor-bearing animals through down-regulation of circulating VEGF-A and reduced expression of VEGFR2, PDGFR, and HIF-1.¹⁷⁴

Toxicity of AuNPs

While a considerable number of experimental work supported the non-toxicity of AuNPs, other research raised concerns about the toxic effects of these particles.^{175,176} Toxicity of AuNPs has been shown to depend on the physicochemical properties of the particles, such as their size, charge, and surface-chemistry.^{177,178}

The majority of toxicity studies revealed that AuNPs with a particle size greater than 4–5 nm in diameter are mostly non-toxic after acute exposures.¹⁷⁷ Nanoparticles smaller than 4 nm in size however become catalytically active and could be cytotoxic.¹⁷⁷ In addition, AuNPs toxicity has been shown to be related to their cellular internalization pathways. Sabella et al showed that AuNPs with stripe-like ligands were taken up by non-endocytic pathways and mainly distributed in the cytosol as opposed to unstructured AuNPs that entered the cells via endocytic pathway and co-localized within lysosomes. This was associated by a greater toxicity of the unstructured AuNPs when compared to the striped ones.¹⁷⁹

Falagan-Lotsch et al evaluated the long-term effects of AuNPs with different shapes and surface coatings under chronic and non-chronic exposure conditions.¹⁷⁷ Viability of the human dermal fibroblasts (HDFs) was not changed between AuNP-treated and non-treated cells in both exposure conditions suggesting AuNPs were generally not cytotoxic to human fibroblasts. However, long-term exposure to AuNPs induced changes in HDFs homeostasis through altered gene expression for genes regulating cell cycle and oxidative stress.¹⁷⁷ Other investigations showed that AuNPs were not cytotoxic to fetal HDFs but affected cell morphology at high concentrations.¹⁸⁰ AuNPs showed low cytotoxicity to hepatic HepG2 cells, though they can disrupt adhesion of the HepG2 cells.¹⁸¹ Bahamonde et al assessed the toxicity of a single intravenous dose of AuNPs in rodents.¹⁸² Mice exposed to the particles developed granulomas in the liver and increased serum levels of the inflammatory cytokine interleukin-18. Such effects were not observed in rats exposed to AuNPs.¹⁸² In terms of AuNP biodistribution and excretion, rats showed greater particle accumulation in spleen and higher fecal excretion compared to mice.¹⁸² Hepatotoxic potential of AuNPs was further investigated using rodents with healthy and stressed liver.¹⁸³ AuNPs were toxic to liver tissues in animals with stressed liver environment but not in animals with healthy liver function. Hepatotoxicity was mediated through increased production of reactive oxygen species, acute inflammation, and increased apoptosis in animals with stressed liver function after exposure to AuNPs.¹⁸³ Toxicity of AuNPs has been assessed using other *in vivo* models as well. Exposure to AuNPs in zebrafish model provided data about their embryonic toxicity.¹⁸⁴ Embryonic toxicity was found to be dependent on AuNPs surface function and charge and ranged from lethal

effects to sublethal malformations.¹⁸⁴ Genotoxicity of AuNPs was evaluated using *Drosophila melanogaster* as an in vivo model.¹⁸⁵ Findings revealed that AuNPs were able to induce genetic mutations, which were further transmitted to *Drosophila* fly progeny.¹⁸⁵ Furthermore, genomic toxicity of AuNPs was evaluated in *Caenorhabditis elegans*. Whole-genome microarray identified significant differential expression of 797 genes of the nematode after exposure to AuNPs.¹⁸⁶ Collectively, toxicity of AuNPs needs further investigation to determine the safety of these formulations for human use. Clearly, the toxic effects are determined by the physicochemical properties of the particles, their biodistribution and cellular uptake, and the cell line or animal model being examined.^{175,187}

Conclusions and future directions

Dysregulation of angiogenesis is the hallmark of multiple pathologic conditions. The search for new drug entities which can modify angiogenic process to treat human disease is highly warranted. Despite the availability of anti-angiogenic drugs in the market, the utilization of these drugs is limited by the lack of therapeutic benefits in specific medical conditions, adverse effect profile, and the potential for the development of drug resistance. AuNPs represent an appealing alternative as therapeutic agents to modify the angiogenic process. In this review, the majority of biomedical applications demonstrated that AuNPs are effective angiogenic inhibitors and could be further modified to facilitate targeted delivery and loading with other drug entities. Most evidence for the effectiveness of AuNPs was demonstrated by pre-clinical studies using cell culture and animal models. It is of paramount importance to test AuNPs in clinical studies to further assess their applicability and to improve and develop these formulations for specific medical applications, which may provide a new approach to overcome the limitations of the currently available angiogenic inhibitors, especially in cancer management.

Despite the potential therapeutic applications of AuNPs, several limitations for their utilization exist. Clearly, the uptake and internalization pathways for AuNPs are highly diverse and are dependent on multiple particle properties and the target tissue. Better understanding of cellular uptake mechanisms and intracellular trafficking would provide great insights into the fate of AuNPs and further their potential therapeutic benefits. In addition, clinical applications for AuNPs require careful

evaluation of their toxicity. Although many studies have demonstrated that AuNPs are biocompatible and non-cytotoxic, there is an increasing demand to examine their toxicity. Therefore, it is critical to develop and/or identify appropriate in vitro and in vivo models to assess the toxicity of these particles.

Abbreviations

AGR2, anterior gradient 2; ALG, alginate; Ang-1 or-2, angiopoietin-1 or -2; AuNPs, gold nanoparticles; CAM, chick chorioallantoic membrane model; CRC, colorectal cancer; CT, computed tomography; CTAB, cetyltrimethylammonium bromide; Den-RGD, dendrimer-arginine-glycine-aspartic acid; EAC, ehrlich ascites carcinoma; ECs, endothelial cells; EGF, epidermal growth factor; EMT, epithelial-to-mesenchymal transition; EPR, enhanced permeability and retention effect; FGF, fibroblast growth factor; HCM, hydrocolloid membrane; HDFs, human dermal fibroblasts; HP, *Hamelia patens*; HRMECs, human retinal microvascular endothelial cells; HUVECs, human umbilical vein endothelial cells; IF- γ , interferon- γ ; IL, interleukin; mCRC, metastatic colorectal cancer; MMPs, matrix metalloproteinases; MPC, monolayer protected cluster; MSOT, multispectral optoacoustic tomography; mTOR, mammalian target of rapamycin; OSCC, oral squamous cell carcinoma; pAuNPs, phytochemically stabilized AuNPs; PBMT, photo-bio-modulation therapy; PDGF, platelet-derived growth factor; PEG, polyethylene glycol, PEG-GNSCs, PEGylated gold nano-semicubes; PIGF, placental growth factor; PVA, polyvinyl alcohol; QAuNP, hybrid quinacrine-gold nanoparticle; RGD, arginine-glycine-aspartic acid peptide; rhES, recombinant human endostatin; siRNA, small interfering RNA; STB, sunitinib malate; TGF- β , transforming growth factor-beta; TIMPs, tissue inhibitors of matrix metalloproteinases; TKIs, tyrosine kinase inhibitors; TNF- α , tumor necrosis factor- α ; TOAB, tetraoctylammonium bromide; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

Disclosure

Figures of this review were created with the aid of Servier medical art, BioRender, and ChemDraw software. The authors report no conflicts of interest, financial or otherwise, in this work.

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