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Gold nanoparticles: opportunities and challenges in nanomedicine

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Importance of the field: Site-specific drug delivery is an important area of research that is anticipated to increase the efficacy of the drug and reduce potential side effects. Owing to this, substantial work has been done developing non-invasive and targeted tumor treatment with nanoscale metallic particles.

Areas covered in this review: This review focuses on the work done in the last few years developing gold nanoparticles as cancer therapeutics and diagnostic agents. However, there are challenges in using gold nanoparticles as drug delivery systems, such as biodistribution, pharmacokinetics and possible toxicity. Approaches to limit these issues are proposed.

What the reader will gain: Different approaches from several different disciplines are discussed. Potential clinical applications of these engineered nanoparticles are also presented.

Take home message: As a result of their unique size-dependent physicochemical and optical properties, adaptability, subcellular size and biocompatibility, these nanosized carriers offer a suitable means of transporting small molecules as well as biomacromolecules to diseased cells/tissues.

Keywords: biodistribution, cancer, diagnostics, drug delivery, drug targeting, gold nanoparticles, photodynamic therapy, thermal ablation

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1. Introduction

Even though our knowledge of cancer biology has increased significantly in the past two decades, cancer is still a major health problem worldwide as the second leading cause of death [1]. Each year, there are > 10 million new cases and > 5 million deaths from the disease [2]. A diagnosis of cancer was previously considered terminal, although, if detected early, the prognosis is favorable. A significant number of cancer patients are asymptomatic until they are in the late stages of the disease. At present, treatments are limited to chemotherapy, radiation and surgery. Owing to inadequate therapies and clinical procedures for overcoming multi-drug resistant cancer, it is vital that new technologies emerge for accurate early detection and treatment of this disease. The primary goal of cancer treatment should result in enhanced therapeutic efficacy with low to minimal side effects. One possible method is to develop nanotechnology for targeted drug delivery. This review focuses on using gold nanoparticles as cancer therapeutics and diagnostic tools.

2. Synthetic methodologies of biological production of gold nanoparticles

Since ancient times, gold has been highly regarded as the 'elixir of life' [3]. In 1925 gold complexes were being used in clinical trials to determine its efficacy to help alleviate rheumatoid arthritis [4]. In recent years, significant effort has been

Article highlights.

- Major benefits of gold nanoparticles as drug delivery agents include: ease of synthesis; and surface easily modified to incorporate an array of ligands for multifunctionality such as targeted delivery.
- Physicochemical properties of the gold core are ideal for photodynamic therapies, contrast imaging and thermal ablation.
- Engineered nanoparticles encompass the capability for early disease detection.
- Advances in the field require a multidisciplinary approach to realize the therapeutic potential of gold nanoparticles.

This box summarizes key points contained in the article.

devoted to developing nanotechnology for the delivery of low-molecular-mass drugs, as well as cellular/tissue delivery of proteins, peptides, or genes [5]. Targeted nanoparticle-mediated drug delivery may be used to direct the particles to specific tissues (minimizing toxicity), improve oral bioavailability and unfavorable pharmacokinetics, sustain a drug/gene effect in the target tissue, solubilize drugs for intravascular delivery, and/or improve the stability of therapeutic agents against decomposition [6-8]. The relative stability of the ligand-gold bonding outside the cell combined with its decreased stability within cells, partially owing to the high intracellular concentrations of glutathione, also contributes to making gold nanoparticles good candidates for drug delivery and drug release [9].

Nanoparticles are a hybrid material featuring an inorganic core typically surrounded by an organic monolayer. The versatility of nanoparticles as components in nanobiotechnology arises from the ability to tailor their size, shape and composition. Quantum mechanics exist at the near-atomic scale in which these nanoparticles are synthesized, where the chemical, electrical and optical characteristics can differ significantly from the bulk solid [10]. Furthermore, slight deviations in nanoparticle size can have radical effects on their properties. The shape of the nanoparticle also influences the particle's behavior. As the ratio of surface area-to-volume increases, the behavior of the surface atoms assumes dominance over those in the particle interior [11]. Finally, the organization of the core material determines the physical properties of the nanoparticles.

The organic monolayer of the nanoparticle, although not as influential in determining the physical properties of the core, is fundamentally important in modifying the interactions of the nanoparticle with its environment. The monolayer acts as a barrier between the nanoparticle core and the environment, and effectively protects and stabilizes the core's integrity. Furthermore, the reactivity and solubility of the nanoparticles are dictated by the chemical nature of the monolayer periphery. For biological applications, the need for solubility in aqueous environments is addressed by designing a monolayer that incorporates biocompatible ligands [12-19]. The monolayer

can be tailored further with more intricate pendant groups to modulate intermolecular interactions of the particle. Most importantly, biologically active components, such as peptides, proteins and DNA, can be conjugated to the nanoparticle monolayer. In effect, the design of the monolayer dictates the chemical behavior of the nanoparticle in a given environment whereas the core governs the physical behavior of the nanoparticle.

Nanoparticles featuring gold cores have been in use for decades [20]. Numerous approaches exist for nanoparticle fabrication, the most simplistic being the reduction of gold salts in the presence of a reducing agent (Figure 1A). This initiates the nucleation of the gold ions, thus forming nanoparticles. To help prevent aggregation, a stabilizing agent is often added during the synthesis process. This method was first reported by Turkevich *et al.* [21] and later improved on by Frens [22]. In this technique, sodium citrate plays a dual role: first as the reducing agent and, subsequently, the stabilizer as it is absorbed onto the surface (Figure 1B). Recent advances in nanoparticle production provide a means to synthesize stable particles that are smaller (2 – 6 nm) and more monodisperse in size and shape. Topmost among these approaches is the Brust-Schiffrin scheme, which produces large quantities of superior quality monolayer-protected gold clusters [23]. In short, gold ions are transferred from an aqueous solution of gold salt into toluene using a phase-transfer agent (tetraoctylammonium bromide [TOAB]). A nonpolar organic thiol is added (usually a thiol-terminated long-chain alkane), followed by the rapid addition of a reducing agent (see Figure 1C). The thiol-mediated capping of the gold cluster ultimately halts agglomeration of the reduced gold atoms. The thiol ligands form a very stable organic monolayer owing to the strong gold-sulfur bonding. The size of the gold clusters can be modulated primarily by altering the gold:ligand stoichiometry.

One key feature of these monolayer-protected gold clusters is that they can be readily modified to incorporate a diverse array of functionalized ligands [24]. Over time, the new ligands are incorporated into the monolayer, whereby the original ligands are displaced into solution (Figure 1D). In this manner, nanoparticles can be endowed with specific attributes such as water solubility, surface charge, or specific recognition. Furthermore, the ligands form a monolayer that is flexible and labile by nature.

3. Targeted delivery *in vitro*

3.1 Size and surface functionality

As mentioned previously, inorganic core shell nanoparticles have an increased impact for biomedical applications such as sensing, imaging and drug delivery [25]. Their success relies on the ability of these particles to target specific cells followed by internalization [26]. It was recently reported that the size and shape of nanoparticles are important in relation to cellular uptake *in vitro*. In a study done by Chan and co-workers, spherical-shaped nanoparticles had an increased uptake over

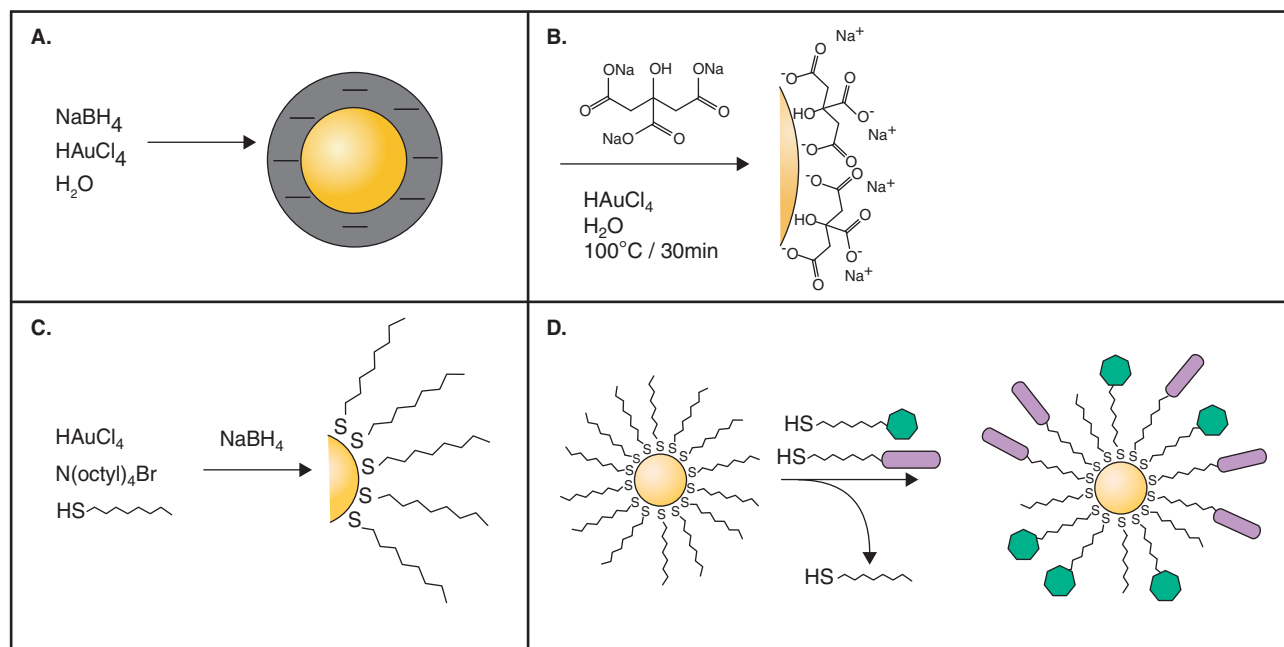


Figure 1. Different schematics for gold nanoparticle synthesis. (A) Basic synthesis of nanogold. (B) Method using the citrate method as both the reductant and capping agent. (C) Brust-Schiffrin synthesis capped with a nonpolar thiol. (D) Murray-place exchange method allowing creation of a multifunctional nanoparticle.

rod-shaped particles [27]. The authors also discovered that the 50 nm spherical gold nanoparticles (AuNPs) had the best internalization of all the nanoparticles studied. Within the last year, the Rotello group has reported using AuNPs with an overall neutral charge to deliver drugs effectively *in vitro* [28,29].

Another important factor of mitigating nanoparticle intake is the surface functionality, allowing for either specific or non-specific interactions with the cellular lipid bilayer. Owing to the overall anionic surface of the cell exterior, cationic nanoparticles are able to be translocated by means of favorable electrostatic interactions [30]. However, it has been reported recently that hydrophobicity can also be important in targeting lipophilic domains [31]. In this report, the authors showed that order and structure of the capping ligands played an important role in how the nanoparticle is internalized into the cell. Gold nanoparticles demonstrating an ordered alternate arrangement of the surface ligands or a 'striped' conformation easily passed through the cellular membrane, whereas a random arrangement of the surface ligands or 'unstriped' nanoparticles did not internalize as readily [31]. In addition, there are other reports where proteins in the serum help facilitate nanoparticle uptake into cells [32].

3.2 Using cell-penetrating peptides

The protective lipid bilayer of mammalian cells acts as an impermeable barrier to most biomacromolecules. However, certain peptides have been shown to be able to penetrate the

membrane and subsequently be internalized. These cell-penetrating peptides (CPPs), which are mainly cationic or amphiphilic, have been used in drug delivery systems as well as gene therapies [33,34]. In one study, a Tat protein-derived sequence was shown to mediate efficiently the nuclear translocation of AuNPs that were < 30 nm in diameter [35]. A recent publication has provided further insight on how surface functionality dictates the subcellular localization of AuNPs *in vitro* [36]. Gold nanoparticles capped with the CCP Tat, a nuclear location signal (NLS), or Antennapedia-Pntn was studied thoroughly using transmission electron microscopy. The CPP-nanoparticle conjugates were transported from the extracellular space through the cell membrane into the cytoplasm, showing their capability to act as a functional unit in drug delivery systems. Furthermore, researchers have successfully demonstrated that particles could enter cells by several different routes. Gold nanoparticles with CPPs appeared to enter the cytoplasm either directly through the cell membrane or by means of endocytosis followed by endosomal release. These results suggest that a combination of peptides to modify the particle surface allows for their release from endosomes into the cellular matrix. In a separate study, Tkachenko tagged a citrate gold nanoparticle with a nuclear-targeting peptide that had been previously modified with BSA [37]. They tested their system against nuclear-tagged nanoparticles in the absence of a BSA monolayer coating in HepG2 cells. Their results demonstrated the hybrid gold particle to be more efficient in nuclear targeting than the particle with peptide alone.

3.3 Surface receptor targeting

The ability of gold nanoparticles to bind strongly with biological molecules is being exploited to target cancer tissues by conjugating the surface of the nanoparticle with suitable biomolecules. These bioconjugated nanoparticles can be used as vectors [38] for delivering drug moieties. As it is well known that epidermal growth factor receptor (EGFR) is often overexpressed in cancer cells, it can be used as a targeting agent for drug delivery [39]. Using this concept along with the intrinsic optical properties of AuNPs, researchers have successfully conjugated anti-EGFR to the surface of gold nanoparticles to demonstrate the detection and imaging of malignant cells [40]. As the conjugated AuNPs have a sixfold greater absorption in cancer cells than normal human cells, El-Sayed *et al.* [40] were able to 'seek and destroy' cancerous cells using a continuous argon laser at 514 nm. More importantly, they were able to demonstrate that after 4 min, 25 W/cm² is lethal to cancer cells compared with 57 W/cm² for normal cells. These studies showed that effective coupling of targeting agents conjugated to gold nanoparticles could be used as viable cancer therapeutics. Other studies done by Patra *et al.* have also successfully used a multifunctional gold nanoparticle tagged with C225 and Gemcitabine, a common drug used in clinics to treat various cancers. This nanoparticle-based drug delivery system was shown to inhibit the growth of pancreatic cancer cells *in vitro* and *in vivo* [41]. Using this strategy, this system can be a viable cancer therapeutic used to target other cancers that overexpress EGFR.

The folic acid receptor is an important receptor for targeted drug delivery owing to frequent overexpression on the surface of malignant cells [42]. Based on their previous work [43], Bhattacharya and co-workers used multifunctional gold nanoparticles as a potential therapeutic for ovarian cancer [44]. The authors were able to show that gold nanoparticles conjugated with thiolated polyethylene glycol (HS-PEG), *cis*-platin and folic acid had a toxic effect on malignant cells (OV-167, OVCAR 5) versus normal cell lines (HUVECs, OSE). The Baker group also utilized the folic acid receptor as a means for targeted drug delivery using dendrimer-encapsulated gold nanoparticles [45,46]. Using KB cells, they were able to show specific binding and internalization within 2 h into lysosomes *in vitro*. A report by Dreaden *et al.* [47] targeted the estrogen receptor alpha using gold nanoparticles conjugated with a tamoxifen derivative. The uptake of the nanoparticle was observed to have a 2.7-fold enhanced potency compared with the free drug.

4. Tumor targeting efficiency *in vivo*

For nanoparticles to advance towards clinical use, it is imperative to have a fundamental understanding of how they behave in the tumor microenvironment in order to optimize their capacity to be efficient therapeutic and diagnostic tools. Owing to the large intercapillary distances and variable blood flow typical of solid tumors, penetration and accumulation of

cancer therapeutics to the hypoxic center of the tumor is often limited [48-50]. However, the proliferating outer shell of solid tumors is often the site of angiogenesis. This highly vascularized region tends to be 'leaky', inducing the enhanced permeability and retention effect (EPR). One common method in designing nanoparticles for passive uptake takes advantage of the hyperpermeability of solid tumors [51-53]. The angiogenic blood vessels in these tissues can have gaps up to 600 nm between adjacent endothelial cells, allowing carriers to extravasate into the interstitial space. This results in the concentration of the carrier at the tumor being 10-fold or higher than the relative levels of the same administered dosage of free drug (Figure 2) [54,55].

Despite the inherent low toxicity of gold nanoparticles [56-59], there have been so far only a handful of studies that have investigated the interaction of gold nanoparticles with tumor tissue. Recently, Perrault *et al.* studied the pharmacokinetics and passive uptake of gold nanoparticles (d = 20, 40, 60, 80 and 100 nm) conjugated with mPEG (molecular mass = 2, 5 and 10 kDa) into tumor tissue [60]. Using CDI mice (n = 4), it was determined that the half-life of the particle is 8 times greater when smaller particles (d = 20 nm, 51 h) are conjugated with 10 kDa mPEG compared with the large particle (d = 80 nm, 6.6 h). Conversely, nanoparticle accumulation into tumors is core size dependent, with the larger particles amassing the best. Their results correlate nicely with previous work with fluorescently labeled dextrans done by Dreher *et al.* [61] showing that small particles are not suitable for accumulation into the tumor matrix owing to their high diffusion rate. Furthermore, it is important to note that accumulation of the smaller core-sized particles into tumors depends on size and their respective half-life compared with the larger particles that are dependent solely on half-life circulation.

In a recent groundbreaking study, *in vivo* delivery of gold nanoparticles loaded with tumor necrosis factor (TNF) to a colon tumor after intravenous injection was demonstrated [62]. Nanoparticle-bound TNF was found to be less toxic and more effective than native TNF, demonstrating the potential of this delivery technique. In these applications, the nanoparticles' role is twofold: a targeting agent as well as a therapeutic. First, the packaging of the desired cargo using nonspecific interactions with the nanoparticle surface confers a certain degree of stability by protecting the cargo from enzymatic degradation [26]. Consequently, the nanoparticles can be targeted at the cellular, tissue, or organ level by integrating molecules into the monolayer such as antibodies (and their fragments), lectins, proteins, hormones, or charged molecules [63].

5. Photothermal/radioactive properties of gold nanoparticles

Some of the pioneering work to design methods for selective cell targeting based on the use of lasers and light-absorbing nanoparticles was done by Pitsillides *et al.* The optical

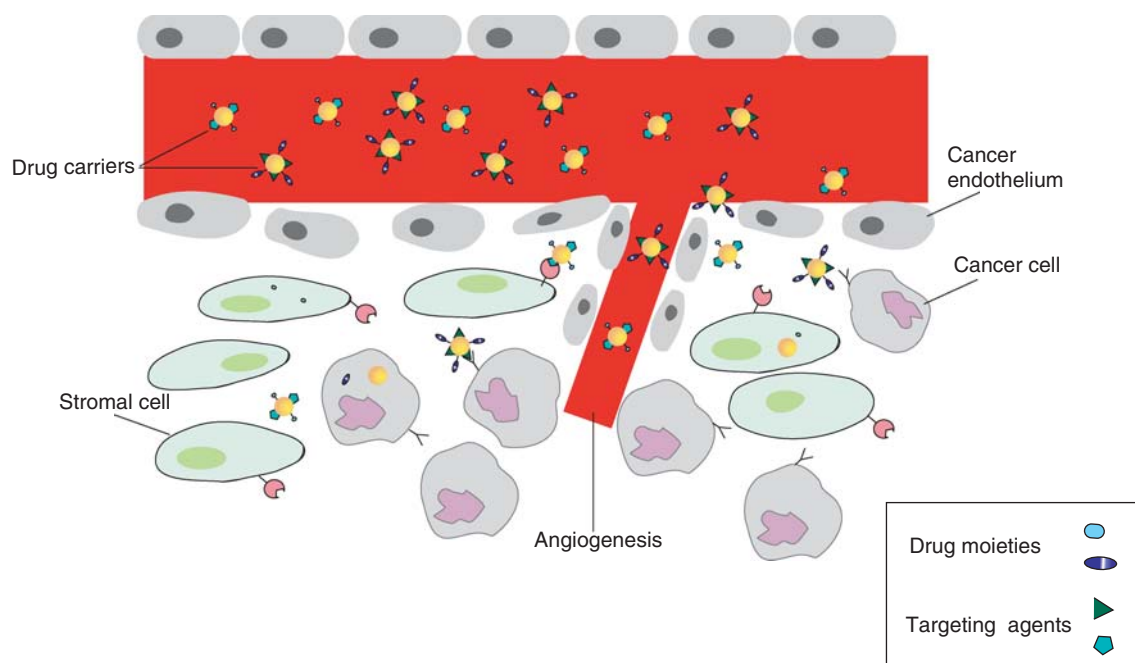


Figure 2. Nanoparticle mediated targeted drug delivery to tumors. The enhanced permeability and retention effect allows easy access of multifunctional nanoparticles for drug delivery to tumors. Since the tumor vasculature is hyperpermeable, gold nanoparticles capped with both a targeting ligand and a drug moiety can easily effuse through the gaps and accumulate inside the tumor tissue. The targeting ligand subsequently binds to its surface receptor allowing for receptor-mediated endocytosis of the nanoparticle. After endosomal escape, the drug moiety can be released from the nanoparticle surface into the cytosol and act accordingly.

properties of gold nanoparticles are based on the surface plasmon resonance and can be used to provide photodynamic therapy (PDT) to diseased tissues. Using short laser pulses focused on monoclonal antibodies conjugated with light-absorbing microparticles and nanoparticles, the researchers developed a new methodology for selective cell targeting and providing highly localized cell damage by photodynamic therapy [64].

Hede and Huilgol have developed hybrid gold-198/199 nanoparticles tagged with prostate cancer-specific bombesin peptide analogues in pursuit of this idea [65]. These radioactive gold nanoparticles can deliver effective therapeutic response for treating prostate cancer. By using gold-198/199 nanoparticles, which emit beta-radiation, a higher therapeutic dose is ensured. This design could also be applied to breast and small-cell-lung cancer cells because the peptide analogue also has a high affinity towards receptors on these cell surfaces.

Surgical removal of tumors becomes difficult once they reach a certain size. However, the work of Chanda *et al.* has recently reported therapeutic efficacy of gum-arabic glycoprotein (GA) functionalized gold nanoparticles [66]. In this study, they administered a single dose of radioactive GA-¹⁹⁸ gold nanoparticles in SCID mice. After 3 weeks, the researchers saw an extraordinary tumor reduction of 82% between the treatment and control groups. Furthermore, there was

minimal radioactive leakage (2%) to various non-target organs and the blood levels of the treatment group returned to a normal baseline, showing high tolerance of GA-¹⁹⁸ AuNPs.

By changing the shape of AuNPs from spheres to rods, the frequency of the surface plasmon band (SPR) of the nanoparticle is modified [11]. One of the advantages of gold nanorods is the duality of the observed plasmon band that is tunable through its aspect ratio. Second, the nanorods can be tailored further owing to the distinctive surface chemistries along their crystal faces. Moreover, the shift of the SPR allows for near-infrared (NIR) absorption at the cross-sections, permitting a deeper penetration into living tissues. Utilizing these properties, von Maltzahn *et al.* have developed nanorods that target and reduce tumors [67]. In this study, PEG-coated nanorods were injected into the tail veins of tumor-bearing mice. The treated mice were then exposed to NIR and after 15 days showed a significant reduction in tumor size. Expanding on their work, the authors tagged the nanorods with SERS reporters and demonstrated their effectiveness at imaging and ablating tumors *in vivo* [68].

Another method used to help reduce tumor size is through radiofrequency. Since the early 1990s, it has been used as a treatment for destroying liver tumors [69]. However, this method does have its drawbacks, such as accuracy, invasive needle placement and damage to non-targeted surrounding

tissues. Owing to these issues, Curley and his research team have recently designed a method using the Kanzius device [70] combined with gold nanoparticles functionalized with the EGFR inhibitor cetuximab (C225) [71,72]. The researchers tested the C225-conjugated gold nanoparticles in Panc-1 (pancreatic adenocarcinoma) and Difi (colorectal adenocarcinoma) cells that express high levels of EGFR. As a control, they used a breast cancer cell line (CAMA-1) that does not express the growth factor receptor. Each cell line was then incubated with C225-AuNPs for 2 h and then exposed to 600 W for 2 min from the Kanzius device. This method proved to be cytotoxic to nearly 100% of the pancreatic and colorectal cells, but hardly any of the cells from the control group were damaged. This technique may establish an accurate and effective treatment for malignant tumors.

A significant attribute of gold nanoparticles is their stability against oxidation and degradation *in vivo*. These attractive properties play a considerable role in the advancement of nanomaterials for use as clinical therapeutic and diagnostic tools [73]. Kattumuri *et al.* have shown that gold nanoparticles functionalized with gum-arabic vectors can be used as a contrast agent in X-ray CT [74]. In addition, the Eck group fabricated pegylated gold nanoparticles conjugated with F19 antibodies to label human pancreatic adenocarcinoma cells that were resected from patients [75]. By using the strong optical properties of gold nanoparticles, they were able to image the actual spatial distribution of the tumor and its subsequent stromal tissue using darkfield microscopy near the nanoparticle resonance scattering maximum (560 nm). This new technique may provide clinicians with facile identification of malignant tissue.

Other reports mention using gold nanoparticles in X-ray radiotherapy enhancement. In one study, mice-bearing EMT-6 mammary carcinomas received a single intravenous injection of gold nanoparticles (7 mg Au/g in tumor) and were further subjected to several minutes of X-ray therapy at 250 kVp [76]. After 1 year, the survival rate was an astonishing 86% compared with the control groups (20% X-ray only, 0% gold only).

6. Using gold nanoparticles to inhibit angiogenesis

As described earlier, angiogenesis is believed to play an important role in the growth and spread of cancerous tissue. This complex process is tightly regulated between a balance of pro-angiogenic growth factors such as vascular endothelial growth factor (VEGF) and anti-angiogenic factors such as thrombospondin-1 (TSP-1) [77,78]. However, during tumorigenesis, pro-angiogenic growth factors are over-secreted to interact with their respective surface receptors, creating the signaling cascade involved in angiogenesis. One method to inhibit angiogenesis *in vivo* is to block the interaction of VEGF with its receptor. Recently, the authors' group has reported that 'naked' gold nanoparticles inhibit VEGF

165-induced proliferation of endothelial cells in a dose-dependent manner [79]. Furthermore, it was shown that the nanoparticles also bind to other heparin-binding growth factors such as basic fibroblast growth factor (bFGF). It has been suggested that the mechanism of inhibition is through the cysteine residues of the heparin-binding domain.

The effect of the 'naked' gold nanoparticles inhibiting angiogenesis was tested *in vivo* using a nude mouse ear model. VEGF 165 was introduced into the ears of nude mice through an adenoviral expression vector (Ad-VEGF). A decrease in edema formation was seen in mice treated with the 'naked' gold versus the sham treated mice. These results were further paralleled in experiments using C3H mice injected with mouse ovarian tumor (MOT) cells. This mouse model has been well characterized and confirmed that VEGF 165 is responsible for ascites formation in human epithelial ovarian cancer [80-82]. After a week of daily intraperitoneal injections, the ascites volume had reduced in the nanoparticle-treated mice compared with the non-treated tumor-bearing mice. Taken together, 'naked' gold nanoparticles inhibit VEGF, reducing angiogenesis and ascites accumulation.

7. Biodistribution

For gold nanoparticles to be effective as a pharmaceutical, it is essential to have a firm understanding of their biodistribution/accumulation in living systems. To achieve this, it is necessary to have proper characterization of the nanomaterials and a good animal model with an appropriate sample size and robust statistical analyses. The biodistribution of drug carriers is often affected by the route of administration [83]. Nanoparticles used as drug carriers tend to have a longer retention time, generally in the local lymph node, compared with the free drug when administered subcutaneously, intramuscularly, or topically [84,85]. However, the biodistribution of the nanoparticles largely depends on surface charge and hydrodynamic radius [86-87]. In work done by the Makino group, particle size was deemed to be important for *in vivo* permeation. Citrate-capped gold nanoparticles of various sizes ($d = 15, 100$ and 200 nm) were prepared using previously published methods [88]. Their studies showed that the 15 nm particle had the highest permeation coefficient. The larger particles showed a lag time of 3 – 6 h. It was also noted that the smaller particles permeated into the deep regions of the skin, whereas the 100 and 200 nm particles remained on the surface. Furthering their work in this area, they injected mice with gold nanoparticles with diameters of 15, 50, 100 and 200 nm [89]. After inductively coupled plasma mass spectrometry (ICP-MS) analysis of the various organs and blood, it was revealed that most of the gold, regardless of size, was present in the liver, lung and spleen. The 15 nm particle seemed to have accumulated the most in all the tissues, including blood, liver, lung, spleen, kidney, brain, heart and stomach. Also, it was discovered that the 15 and 50 nm particles were able to cross the blood-brain barrier, whereas the 200 nm particle

Table 1. Possible targets for nanoparticles [98].

Target	Function
VEGF	Angiogenic factor
Flt-1, Flk-1	VEGF receptors
CD105	TGF- β superfamily receptor
PSMA	Glutamate carboxypeptidase
TEM8	Adhesion molecule
CTGF	Growth factor
$\alpha_v\beta_3$ integrin	Adhesion molecule
MMPs	Endopeptidases
uPa	Serine protease
uPAR	uPa receptor
Tenascin-C	Mitogenic and adhesive effects
FAP α	Serine protease
CAIX	Carbonic anhydrase

showed a very minute presence in the organs including blood, brain, stomach and pancreas. Geertsma and co-workers illustrated that 10 nm gold nanoparticles were widely dispersed in various organs, whereas the larger particles were detected only in the liver, blood and spleen [90].

In a study done by Balogh *et al.* [91], five types of dendrimer-encapsulated gold nanoparticle ($d = 5 - 22$ nm) bearing positive, negative or neutral surface charges were injected into mice. After killing, the various organs, blood and excrement were analyzed for gold content. The researchers concluded that size and surface charge of the nanoparticles affect biodistribution, with the smallest positive particles accumulating in the kidneys and larger ones accumulating in the spleen, liver, lungs and heart. Work done in the Brandau lab systematically analyzed the effect of size and ionic ligands of monodisperse 1.4 and 18 nm gold nanoparticles after two routes of administration: (i) intratracheal instillation into the lungs (IT); and (ii) intravenous injection into the tail vein of rats [92]. Their results indicated that the 1.4 nm particle translocated through the respiratory tract compared with the 18 nm particle. However, after intravenous injection, the accumulation pattern in other organs revealed a different pattern. The authors hypothesized that proteins and cells modify the gold nanoparticles during the translocation process.

To design a 'biocompatible' nanoparticle monolayer, it is necessary to incorporate a chemical functionality that masks the hydrophobic nature of the monolayer core, such as poly (ethylene glycol) (PEG). PEG has been used extensively to passivate surfaces, as it fundamentally resists nonspecific interaction, especially of biomolecules [93-95]. Work done by Zhang *et al.* [86] investigated the pharmacokinetics and biodistribution of various PEG-AuNPs in nude mice. Their studies revealed that thioctic PEG produced a more stable nanoparticle compared with HS-PEG in the presence of dithiothreitol. Based on these observations, the researchers synthesized AuNPs with high- (5000 kDa) and low-molecular-mass (2000 kDa) thioctic-terminated PEG. From this array, it was observed that the 20 nm AuNPs tagged with

PEG-5000 showed the lowest uptake in the reticuloendothelial system and the longest half-life. Compared with the 40 and 80 nm PEG-5000 AuNPs, the 20 nm particles also showed significantly higher tumor extravasation.

8. Conclusion

Nanotechnology can play an intimate role in individualized medicine, with potential enhancements increasing the affinity of the nanoparticle for cancerous cells and amplifying the uptake of diagnostic and therapeutic moieties into diseased cells with greater efficiency. Although more research is necessary, it has had an impact on our understanding of biology by introducing unique approaches to numerous fields of research, ranging from bioanalysis to imaging. The term nanobiotechnology encompasses a myriad of interdisciplinary technologies based on the integration of molecular-scale synthetic materials with biological building blocks, as well as utilizing nanoscale materials to influence biological components. As systems at the atomic, molecular and supramolecular scales (1 – 100 nm) [96] show fundamentally new properties resulting from their small structure, new molecular architectures can be developed with a high degree of precision and flexibility. Utilizing these principles, nanoparticles can be tuned to enhance their affinity for different cancer/diseased cells and effectively facilitate the advancement of diagnostic and treatment methods.

9. Expert opinion

Gold nanoparticles have materialized as promising candidates for drug delivery systems and cancer therapeutics. The active application of AuNPs centers primarily on two key aspects that make them suitable for use in biological systems: the intrinsic properties of the gold core; and the ability to tailor the functionality of the surface. In general, current applications of nanoparticles have focused primarily on the detection of biological molecules or events. This has been accomplished with great success, as illustrated above and highlighted in recent reviews [11], and continues to be a chief aim of nanoparticle research and funding. However, more research needs to be focused on optimizing the design of nanoparticles as multifaceted vectors to treat diseases such as cancer. For example, the nanoparticle scaffold can be decorated with a pro-drug, a targeting moiety and an imaging agent. Thus, this type of nanoparticle will have multifunctional activities for a more effective cancer treatment that could easily be translated into the clinics. Furthermore, fundamental studies to understand the molecular interactions of nanoparticles with their target cells (normal as well as malignant) remain largely unexplored. Such a fundamental understanding of nanoparticle-cell interactions is important in nanotechnology for cancer therapeutics as well as cancer detection and diagnosis.

There are certain critical questions in nanoparticle design and application that need to be addressed before further use in the clinics. One important problem in potential therapy

Table 2. Current nanomedicine candidates for cancer treatment [98,99].

Product	Structure	Target/action	Phase
HuJ591	Nanoparticle aptamer bioconjugate	PMSA	Preclinical
Aurimmune	PEGylated colloidal gold particles	Solid tumors	Phase I
DF1	Dendritic fullerene	Chemoprotection	Preclinical
AuroLase	Gold nanoshell	Head and neck cancer	Preclinical

is that toxicity must be examined carefully. Even though it has been reported that gold nanoparticles are inherently non-toxic, it is important to discern the toxicity of the nanoparticle core and that of its capping ligands. Some toxicity may be specific to certain ligands. For example, cationic ligands clearly cause moderate toxicity *in vitro* [97]. Equally important is considering how the conjugated ligands may change the pharmacokinetics, biodistribution and eventual side effects. Similarly, packaging technology needs to be optimized if the obstacles of immunogenicity and tumor penetration are to be overcome.

Owing to chemoresistance and the heterogeneity among cancer cells, certain therapeutics may not be successful for

every patient. One strategy to overcome tumor heterogeneity is probably to target stromal cells (Table 1). By tagging nanoparticles with stromal antagonists, it is possible to increase greatly the effectiveness of anticancer therapeutics. Further investigation is warranted to reveal new molecular targets that are expressed only in the tumor microenvironment to aid the targeting of nanoparticle-based therapy. Cancer stem cells (CSC) or cancer-initiating cells (CICs) are also important candidates for drug targeting. By eradicating CSCs/CICs, chemoresistance and tumor reoccurrence may be eliminated.

At present, there are several candidates at different states of preclinical development (Table 2). However, characterization of systemic performance of gold nanoparticles *in vivo* is essential in order to advance the future of nanomedicine. Each multifaceted nanoparticle system is unique and must be evaluated individually. At this point, wide inferences should not be made as to how various nanoparticles behave and interact in biological systems. Systematic studies on toxicity, pharmacokinetics and efficacy need to be carried out under precise conditions before going to clinical trials.

Declaration of interest

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