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The Development and Applications of Gold Nanoparticles for Cancer Therapies: A Review

Mark Nashi

Signal Mountain Middle-High School, 2650 Sam Powell Trail, Signal Mountain, Tennessee, 37377, USA; mark_nashi@yahoo.com
Mentor: Lumiere Education

ABSTRACT: The field of gold nanoparticle research is becoming more prevalent as it is recognized for its potential in cancer research due to its growing effectiveness in treating tumor cells. This review summarizes the most recent studies on using gold nanoparticles in cancer therapies by examining their effect on photothermal therapy, drug delivery, and diagnostic imaging. Additionally, the methods of synthesis and its cell interactions were also studied by examining its protein corona formation, bioavailability, cytotoxicity, and cell signaling (especially the Wnt/β-catenin pathway). Its safety and biocompatibility and its current state with FDA approval were also considered. Future applications, especially green synthesis methods, were also discussed, as the safety improvement would contribute mainly to approving more clinical trials. While gold nanoparticle research in its application to cancer therapies is relatively modern, the studies in this review highlight its growth and promising capability in the biomedical field.

KEYWORDS: Materials Science, Nanomaterials, Gold nanoparticles, Synthesis, Cytotoxicity.

Introduction

This review summarizes the usage of gold nanoparticles in cancer therapies. This means that while this review summarizes the many studies on gold nanoparticles and their effects, it also connects the development of gold nanoparticles with the results of these studies to enhance the current discussions on this topic. It also condenses the synthesis, interactions, usages, and disadvantages of gold nanoparticles to provide an overview of the development of gold nanoparticles in the biomedical field. With this, it references how gold nanoparticles could be utilized in the future and the types of cancer they could address by providing the results of many different *in vivo*, *in vitro*, *ex vivo*, and even Monte Carlo simulation studies. I believe that readers will be able to obtain a new understanding of the future of gold nanoparticles in cancer therapies. It will allow them to visibly see the direction of gold nanoparticles through the many condensed results in this literature review.

While gold nanoparticles are perceived as a relatively new concept, their synthesis and usage date back to the Romans; for instance, the Lycurgus Cup in the 4th century utilized 50-100 nm nanoparticles to create dichroic glass.[1] Medieval church windows also synthesized gold and silver nanoparticles. While these do not relate to the applications of gold nanoparticles in cancer research, they indeed show their effect on the field.

Some note, however, that the field of modern nanoparticle synthesis began with Michael Faraday.[2] Faraday reduced an aqueous solution of a gold compound with a reducing agent in a two phase system. The results turned the color of the aqueous solution from yellow to ruby gold. The colloids produced were extremely stable and resulted in sizes from 4 nm to 8 nm.[3]

It must be noted that gold nanoparticles are not the only metal used for nanoparticles. Other metallic nanoparticles include magnesium, titanium, copper, zinc, silver, and platinum.[4] Gold nanoparticles are utilized rather than other materials mainly due to their physical and chemical properties in photothermal therapy, drug delivery, and biodetection.[5] A large part of their usage is also in their safety. They are considered the safest nanoparticles for these therapies; thus, nanoparticle-based medicines have a higher chance of approval.[5,6]

Discussion

Synthesis of Nanoparticles:

- ***Effects of Size and Shape:***

Nanoparticles are synthesized based on various factors. The size of nanoparticles can drastically impact their applications and toxicity. For instance, in a study that examined amphiphilic gold nanoparticles, the nanoparticles that were synthesized to be about 14 nm were typically utilized for photothermal therapy.[7,8] The size was chosen because nanoparticles larger than 20 nm typically amplified SERS signals, and the study aimed to minimize the amount of background signals for individual nanoparticles to create a contrast between the applied plasmonic vesicles and the gold nanoparticles themselves.[8] Meanwhile, nanoparticles synthesized as an agent for cancer imaging typically range from 16.4 nm to 20.3 nm.[7] These results were found through a study run by Muthu in 2015.[9] In terms of toxicity, smaller nanoparticles have usually led to more toxicity in *in vivo* studies, while larger nanoparticles, those over 50-100 nm, were non-toxic.[7] Additionally, shape has a considerable impact on the applications of nanoparticle technology. For example, gold nanorods are typically synthesized to detect tumors and for photodynamic therapy. Meanwhile, nanoshells are synthesized for simultaneous cancer therapy. It should be noted that while nanoparticles can be synthesized in various shapes, nanorods are often preferred due to their "easy synthesis and large surface to interact with light per unit of volume".

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However, nanoparticles can be developed into other shapes. Nanocages are typically synthesized to hold smaller nanoparticles with magnetic properties. Gold nanostars are typically synthesized as substrates for surface-enhanced Raman scattering (SERS).[10,11]

- ***Approaches to and History of Synthesis:***

The approaches to synthesizing nanoparticles are boiled down to the "Top-Down" and "Bottom-Up" approaches. The "Top-Down" approach is the breakup of a more extensive, bulk material into tiny fragments that eventually make nanoparticles.[12] Conversely, the "Bottom-Up" approach begins with metallic salts or molecular seeds that condense into clusters which eventually form nanoparticles. The precursors used in the "Bottom-Up" method usually are reduced by reducing agents such as sodium borohydride, hydrazine, and citrate, which initiates nanoparticle nucleation.[7] The "Bottom-Up" approach is more cost-effective and can create smaller nanoparticles with absolute precision.[13,14] There is also complete control over synthesis with the "Bottom-Up" method, and the energy loss in synthesis is much higher with the "Top-Down" method. As a result, the "Bottom-Up" method is generally preferred when synthesizing gold nanoparticles.[7] This is especially true in applications that require small nanoparticles with little to no deviation in size, such as photothermal therapy.

The synthesis of nanoparticles can be traced back to the chemical methods of John Turkevich. Turkevich notably taught at Dartmouth College before he took on research at Princeton University.[15] He became a founding member of the International Catalysis Society in 1996 for his work in chemistry. Turkevich is famously known for developing a commonly used method for the chemical synthesis of nanoparticles.[12] Although his study was conducted in 1951, his method is still used to synthesize nanospheres. Therefore, evaluating this method is necessary to obtain a foundation for one of the nanoparticle synthesis methods. In this study, a sodium citrate sol was prepared by boiling 95 mL of a chloroauric acid solution containing 5 milligrams of gold.[16] A 1% sodium citrate solution of 5 mL was added, resulting in spherical gold colloids forming. More specifically, the gold colloids were about 20 nm in diameter. A study by Kimling not only revisited this technique, but also explored two other approaches.[17] One of these was UV irradiation of 2 mL gold hydrochlorate solution with amounts of citrate while the other was the reduction of ascorbic acid at room temperature with the rapid mixture of a 1 mL gold hydrochlorate solution. It must be noted that while Turkevich's method is now commonly known, it was not the first nanoparticle synthesis method. As mentioned before, Faraday preceded all other nanoparticle researchers in the creation of his own sol. Turkevich notes this in his study as Faraday's method (1857) was investigated, which created nanoparticles that had a diameter of 5 nm. However, it must be noted that the common Turkevich method of reducing the tetrachloroaurate ion through citrate is not the only synthesis method.

While the reduction of the tetrachloroaurate ion through citrate synthesizes nanospheres, seed-mediated growth is often used to synthesize nanorods.[12] In seed-mediated growth, gold salts are reduced in the presence of seed particles through reducing agents. More specifically, a growth solution is created utilizing cetyltrimethyl ammonium bromide (CTAB), HAuCl4, ascorbic acid, and silver nitrate (although its function in nanorod synthesis needs to be understood).[18,19] For the creation of nanorods, CTAB concentration, is increased from $1.6x10^{-2}$ *M* to $9.5x10^{-2}$ *M*. This growth solution will allow the creation of gold nanorods through the reduction of gold ions (III). While reduction with citrate created the widely used nanospheres, other reducing agents (such as CTAB or polyol) allow for other applications of nanoparticles through the shaping of rods. A schematic can be seen below of the seed-mediated growth of nanorods.[20]

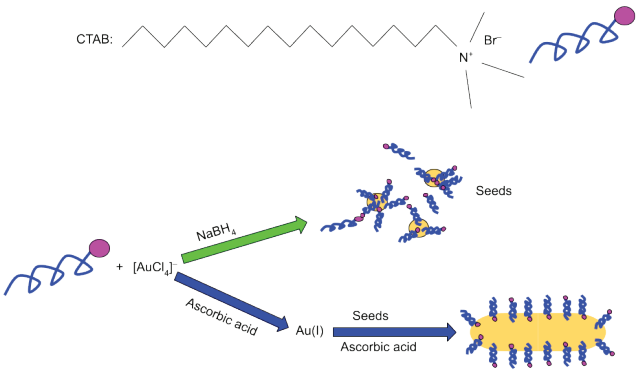

Figure 1: Schematic of the seed-mediated method that produces gold nanorods (Adapted from Chen *et al.*, 2013).[20]

- ***Applications of Synthesis:***

Gold nanoparticles are synthesized for different usages and applications. In fact, a study by Song and his colleagues expanded the synthesis of gold nanoparticles for chemotherapy. In this *in vitro* study, Doxorubicin (DOX), a chemotherapy drug, was loaded into plasmonic vesicles by creating a pH gradient.[8] For clarification, the study depicted plasmonic vesicles as pockets assembled by amphiphilic gold nanoparticles that can store drugs such as DOX. Both pH 7.4 and pH 10 samples were prepared, and due to their higher fluorescence, the pH 7.4 sample was used for cellular imaging. The vesicles were directed by the HER2 antibodies and clustered in the cell membrane. Eventually, the DOX successfully penetrated through the nucleus. While this study targeted breast cells, other cancers require different antibodies. Epigallocatechin gallate, a phytochemical component of green tea with redox properties, was utilized to synthesize radioactive gold nanoparticles for a therapeutic agent in prostate cancer.[21] Epigallocatechin gallate was used as it had promising results and could treat brain, prostate, cervical, and bladder cancers. Specifically, it was used to target tumors in the study as the many cytotoxic drugs that were undergoing clinical trials at the time of publication were not clinically efficient or safe to treat prostate cancer. An *in vivo* study by Safwat supports the fact that epigallocatechin gallate could be used to efficiently treat tumors. Green synthesis was utilized to create epigallocatechin gallate-loaded gold nanospheres with colloidal stability to treat tumors in ehrlich tumor-bearing mice.[22] This study confirmed the hemocompatibility of these nanospheres (hemolysis percentage was below 2%), while also confirming the decline in

tumor volume with the usage of these nanospheres. Safwat reports that it was perhaps the small size, negative charge, and sustained drug release of the nanoparticles that allowed for the accumulation of the drug in the tumors and led to the decline of the tumors.

While nanoparticles could be synthesized to treat cancers, nanoparticles can also be applied before treatment. An example of this is the usage of nanoparticles in diagnosing cancer. In El-Sayed's study, Turkevich's method was used to synthesize nanoparticles for molecular biosensor techniques to diagnose cancer.[23] Surface plasmon resonance was utilized, and it was found that the SPR absorption spectroscopy with colloidal gold nanoparticles was useful in diagnosing cancer. While these nanoparticles were spheres, nanoparticles are also synthesized in other shapes for different applications. Nanorods are typically synthesized by the seed-mediated method to serve two purposes: detecting cancer and photodynamic therapy. However, it must be noted that these are not the only two purposes nanorods serve, as the effectiveness of nanorods in cell interactions and drug delivery will be discussed later in this review. For cancer imaging, chemical synthesis using a sodium borohydride solution was used (though, again, this is not the only application of this reducing agent).[24] The fact that various synthesis methods can lead to differing applications shows the versatility of gold nanoparticles in cancer therapeutics.

Nanoparticle and Cell Interactions:

- ***Protein Coronas:***

Gold nanoparticles usually interact with proteins, which adsorb onto the nanoparticles themselves, forming protein coronas. These protein coronas can change the nanoparticles' properties, manifesting into a problem for gold nanoparticles targeted for cancer cells.[25] However, some argue that these protein coronas may be desirable. Protein coronas can reduce cytotoxicity, harmful interactions with the immune system, and help with cellular internalization.[26] In fact, some researchers have introduced the idea of a "personalized protein corona" to produce personalized nanoparticles for cancer therapies. Regardless, the formation of protein coronas can be seen in an *in vivo* study comparing differing sizes of gold nanorods and nanostars.[27] These nanoparticles were injected into CD-1 mice via their tails. Plasma was then recovered. The results found that while more protein adsorbed onto 70 nm nanostars, the number of varying proteins adsorbed was higher on 40 nm nanostars. The 40 nm nanostars additionally had a greater total of varying proteins when compared to 40 nm nanorods. However, the opposite effect was observed for 70 nm nanostars and nanorods when comparing the total number of differing proteins. While the size and shape of the nanoparticles certainly impact the formation of a corona, the form a nanoparticle takes can also significantly affect these corona formations. A study examined the development of these protein coronas with three nanoparticle forms: "spiky," "mid-spiky," and spherical.[28] These forms indicated the nanoparticles' shape and surface roughness. For instance, spiky nanoparticles had the most surface roughness while spherical nanoparticles had the least surface roughness. Both *in vitro* human umbilical vein endothelial cells (HUVEC) and *ex vivo* swine vessels were utilized in this study. The results found that spherical nanoparticles resulted in higher coronal proteins due to the higher available surface area of the gold nanospheres. However, the "spiky" nanoparticles were found to have a more significant amount of immunoglobulin bound to the surface than the "mid-spiky" or spherical nanoparticles. Immunoglobulin can result in immune recognition by macrophages. This presents a problem as the phagocytosis of these nanoparticles can lead to less bioavailability. Additionally, the "spiky" nanoparticles induced a greater endothelial leakiness. The *ex vivo* experiment confirmed this while further citing that even the "mid-spiky" nanoparticles caused endothelial leakiness comparable to the "spiky" nanoparticles. Not only can the "spikiness" of a nanoparticle alter its protein corona, but the shape and size can also significantly impact it. Protein coronas are not the only challenge gold nanoparticles face once injected.

- ***Bioavailability and Cytotoxicity:***

Gold nanoparticles face bioavailability challenges when targeting a specific body region, and it has been shown that the size of nanoparticles affects this. For instance, an *in vivo* study evaluated differing sizes and surface chemistries to determine the bioavailability of gold nanoparticles in zebrafish.[29] Gold nanoparticle diameter sizes of 10, 20, 40, 80, and 100 nm were utilized alongside modified 80 nm nanoparticles that were divided into three groups of surface chemistries: tumor necrosis factor (TNF), N-Hydroxysuccinimide (NHS), and polyethylene glycol (PEG). Additionally, to further analyze the size effect, this study used a single exposure study using 10, 20, and 100 nm nanoparticles at a concentration of 2 mg/mL. For uptake, embryo larvae were placed in a 24-well plate and exposed to nanoparticles for 72 hours. Zeta potentials are usually described as the potentials at the shear plane of a particle.[30] The negative zeta potentials of the nanoparticles were analyzed, and conclusions were made based on neutrality (the more neutral zeta potentials indicated unstable nanoparticles).[29] The instability of the nanoparticles was defined as the tendency of aggregation. For instance, nanoparticles with more neutral zeta potentials were more prone to aggregation than the nanoparticles with more negative potentials. The results found that the order of stability from most to least stable was 80 nm, 10 nm, 100 nm, 20 nm, and 40 nm nanoparticles. This is especially interesting as it confirms the study's previous statements that 80 nanometers are considered the usual size for nanoparticles. This only furthers the point that the stability of a nanoparticle and its surface charge varies based on certain sizes of nanoparticles. The order from most to least stable for surface chemistry was TNF, PEG, and NHS. Uptake and accumulation for all nanoparticles were found in the pronephric tubules and the gut. This excludes 100 nm nanoparticles, as there was no size uptake due to poor absorption. This suggests that while a larger size might lead to less cytotoxicity,[7] the bioavailability might suffer due to the nanoparticle being too large. The lack of bioavailability may have come from a relative "lack of ingestion" or rapid elimination by the zebrafish from the gut.[29] However, it must be noted that this study analyzed the embryo of zebraf-

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ish, and thus, there was no injection process. This may prove to be a challenge when comparing human test subjects to the results found in this study, especially when considering cancer treatment. It must also be noted that nanoparticles modified with NHS were found to have the lowest uptake. In contrast, those modified with PEG or TNF increased the uptake and retention.[29] 40 nm and 80 nm nanoparticles were found to be most readily taken up by the tissues. In comparison, 10 nm and 20 nm nanoparticles were less readily accumulated. The uptake of gold nanoparticles being size-dependent was also confirmed by a study by Qui and his colleagues in which shorter nanorods were more internalized than longer nanorods.[30] Not only did size matter in this experiment, but surface chemistry also mattered as nanoparticles coated in polystyrene sulfonate (PSS) were not as easily internalized as those coated with poly(dia-llyldimethyl ammonium chloride) (PDDAC). This could have been attributed to the fact that PSS-coated nanoparticles had negative zeta potentials (thus, making them negatively-charged) while PDDAC-coated nanoparticles were positively charged. Size-dependency was also confirmed in a study done by Xia. Uptake in HepG2 cancer cells increased proportionally with respect to the size of nanoparticles.[31] For Windell's study, clearance rates with gold nanoparticles were also particularly interesting, with the 1 mg/L and 2 mg/L groups having reduced levels of fluorescence after 24 hours by 25% and 16%, respectively, and all groups having reduced levels of fluorescence after 96 hours by 28% or more.[29] While this study analyzed gold nanoparticles through absorption into zebrafish, another study analyzed the oral bioavailability of gold nanoparticles in mice. The study done by Alalaiwe acknowledged that intravenous injections are often generally used. Still, it is also acknowledged that the clearance from the reticuloendothelial system decreases the nanoparticle's circulatory half-life, and the drug is often eliminated.[32] However, it also noted that oral transport leads to uptake by other cells in passive transport. While both methods cause issues in bioavailability, this study looked to see if surface chemistry could affect oral bioavailability. The authors of this study previously investigated PEG as a possible modification; however, bioavailability was too low to produce any significant effect. This study created nanoparticles with a diameter of 3 nm, and mice received these nanoparticles orally. One group received 8 mg/kg body weight, and the other received 0.8 mg/kg body weight of gold nanoparticles. While the chitosan-coated nanoparticles resulted in only 2.46% bioavailability, it should be noted that this value is 25-fold higher than the PEG coatings. Still, this bioavailability is relatively low, suggesting that oral methods are not advanced enough. When discussing bioavailability, drug delivery often comes up as a viable concern. For instance, curcumin has a wide range of properties in cancer therapy; however, due to its hydrophobic nature leading to its insolubility in water, its bioavailability is especially low.[5] Gangwar's study examined the effect gold nanoparticles coated with polyvinyl pyrrolidone (PVP) can have on increasing the solubility of curcumin. To synthesize nanoparticles, this study utilized chloroauric acid and trisodium citrate as metal precursors and reducing agents to create 13 nm nanoparticles. The results found that curcumin would attach to the PVP-coated gold nanoparticles through hydrogen bonding to the enolic hydroxyl group. PVP then would have the hydrophilicity to make curcumin soluble in water and, therefore, bioavailable. While no cytotoxicity was found for concentrations of 10 μg/ml, cytotoxicity was enhanced past this concentration. While the study states that this could be beneficial to some extent as it could target tumor cells, it is always important to note that any cytotoxic effect should be treated cautiously. This leads to the necessary discussion of cytotoxicity.

While Gangwar's study mainly dealt with cytotoxicity in reference to dosage,[5] many other factors play into the effect of cytotoxicity with gold nanoparticles. The study done by Qui, as mentioned before, examined these factors that surrounded gold nanoparticle cytotoxicity. Gold nanorods were synthesized with four different aspect ratios and three coatings: cetyltrimethylammonium bromide (CTAB), polystyrene sulfonate (PSS), and poly(dia-llyldimethyl ammonium chloride) (PDDAC).[30] It should be noted that this study used human breast adenocarcinoma cell lines for all experiments. Zeta potentials were examined, and it can be assumed that all nanorods were relatively stable as they all stayed above 30 mV and under -30 mV.[30,33] Over time, they also seemed to affect the cytotoxicity for CTAB.[30] As time increased, the cell viability decreased; however, it must be noted that this was not the case for PSS and PDDAC-coated nanoparticles. In the experiment, there was a common consensus that without serum, the cytotoxicity of gold nanorods would increase drastically. This study also concludes support of the argument that surface chemistry does result in an effect of cytotoxicity. When CTAB gold nanorods are coated with PSS and PDDAC as additional coating, cytotoxicity is reduced to negligible amounts. However, this source does acknowledge some studies that disagree with these findings. For instance, in Windell's study of zebrafish, there seemed to be no indication that gold nanoparticles caused cytotoxicity, regardless of size or surface chemistry.[29] Another study by Chithrani analyzed nanoparticles with 14, 30, 50, 74, and 100 nm diameters.[34] The results found that the maximum uptake was at 50 nm; however, there was no indication of cytotoxicity by gold nanoparticles. In this study, the cellular uptake would increase for 2 hours before plateauing for the next 4-7 hours, suggesting time had a limited effect on uptake and, therefore, toxicity. However, shape did determine the uptake of nanoparticles, as nanospheres experienced more uptake than nanorods. Maximum uptake at 50 nm was also seen in the study done by Xia.[31] Cytotoxicity was also seen with the 5 nm nanospheres as apoptosis was induced with the Hep2G cancer cells and necrosis was seen with the L02 cells. While this review has discussed protein coronas, phagocytosis, drug delivery, and uptake, it has not addressed the nanoparticle's role in signal pathways.

- ***Interactions in Intracellular Pathways:***

Gold nanoparticles can interact with signal pathways such as MAPK and Wnt/β-catenin. This section will examine gold nanoparticles and the Wnt/β-catenin pathway. In the Wnt/β-catenin pathway, β-catenin is degraded by the destruction complex, which comprises different kinases. In the activation of Wnt signaling, however, the nucleus

uptakes β-catenin, creating target genes that may further tumor progression.[35,36] One study examined the effects of gold nanoparticles on this signal pathway, explicitly looking at promoting human adipose-derived mesenchymal stem cells (hADMSC) into osteoblasts. The average diameter of these gold nanoparticles was about 17 nm, and these particles were relatively stable at a Zeta potential of about 42 mV.[37] There was no effect on the cell viability of hADMSC for concentrations up to 10 ppm. This study found that positively charged gold nanoparticles did stimulate hADMSC into osteogenesis as alkaline phosphatase (ALP), a regulator in the process of bone marrow differentiation, increased with the gold nanoparticle group. Choi's study suggests that nanoparticles not only interfere with cell signaling but can significantly alter the fate of cells. A study done by Liang also examined the role of gold nanoparticles in osteogenic differentiation through hydroxyapatite-loaded gold nanoparticles.[38] Both gold nanorods (80-100 nm in length and 20-30 nm in width) and gold nanospheres (4.7 nm in diameter) were utilized for the experiment. Not only did the nanoparticles promote early stage osteogenic differentiation and the mineralization of hMSCs, but they also enhanced the mRNA expression of osteogenic-related genes and enhanced β-catenin in hMSCs. The Wnt/β-catenin pathway also affects the proliferation of human periodontal ligament stem cells (hPDLSC), and a study done by Li examined how 60 nm gold nanoparticles promote these cells.[39] Li's results found that gold nanoparticles promoted the proliferation of hPDLSC, and negatively charged gold nanoparticles would reduce the ALP activity in osteogenesis. Ultimately, it is clear that nanoparticles play some significant role in cell signaling.

Photothermal Therapy Using Gold Nanoparticles:

As previously mentioned, one of the many applications of gold nanoparticles is in photothermal therapy. This therapy is used to damage tumors by converting light energy into near-infrared radiation (NIR).[40] Near-infrared light is an important term as it is utilized for photothermal therapy due to its penetration of the human skin without damaging skin cells or other tissues.[41] Cell necrosis is another important concept, as there is an important difference between cell death and necrosis. While higher temperatures in photothermal therapy lead to more cell death, there are problems when it causes cell necrosis.[42] There are usually two categories for photothermal therapy: mild temperature (41-49 degrees Celsius) and high temperature (over 50 degrees Celsius). High temperatures can eradicate tumors, but this could lead to intracellular constituents released into the extracellular milieu, which would cause inflammatory responses. However, mild temperatures, while they can cause cell death, are hindered due to the heat shock response in cells. It should be noted that cell death is induced at 41 degrees Celsius, and necrosis is induced at 46 degrees Celsius or higher.[43] Xia and her colleagues used liposome-templated gold nanoparticles to investigate the overexpression of heat shock proteins (HSPs), which limit the effect of heat in cells, in photothermal therapy using tumors in mice.[42] It was found that as the concentrations of the liposome-templated gold nanoparticles increased, the temperatures also increased. A similar trend was observed with power, and it was also observed that as temperature increased from 42 to 55 degrees Celsius, the tumor inhibition ratios would also increase. It must be noted, however, that the 50 and 55 degrees Celsius temperatures caused cell necrosis, and as a result, 47 degrees Celsius was chosen as the optimal temperature for photothermal therapy. Achieving this temperature, therefore, should be the focus of photothermal therapy. Another study evaluated this balance between the induction of cell death and necrosis. A study done by Niu examined the genetic and photothermal therapy potential for gold nanorods coated in polydopamine (PDA).[44] Gold nanorods typically exhibit two extinction peaks: transverse and longitudinal surface plasmon resonance (TSPR and LSPR, respectively). LSPR peaks are significant as they improve photothermal conversion efficiency. This was examined in the photothermal aspect of this study. To achieve combination therapy, this study attached microRNA-192, a genetic drug, to nanorods coated in PEG and PDA and examined under ultrapure water. These gold nanorods were synthesized through seed-mediated growth and were ultimately approximately 56 nm long and 16 nm in diameter. The concentrations for gold nanoparticles were as follows: 0.05, 0.03, 0.015, 0.0075, and 0.00375 mg/mL. The results showed that a higher concentration of gold nanorods would lead to a higher temperature, as the 0.05 mg/mL concentration caused a temperature rise of 42 degrees Celsius within 10 seconds and 89 degrees Celsius after 10 minutes. This would not only be enough to cause cell death but also dangerous cell necrosis. The laser power also contributed to a higher photothermal conversion effect as the maximum laser power studied (2.0 W/cm^2) caused a maximum temperature of 91 degrees Celsius. It is clear that while a desired amount of temperature rise is produced after a short period of time, utilizing gold nanoparticles for longer times should be treated with care.

The various shapes of nanoparticles can also affect the efficacy of photothermal therapy. Previously, plasmonic vesicles were discussed in this review as being able to transport both gold nanoparticles and doxorubicin. These plasmonic vesicles unsurprisingly have applications in other therapies, including photothermal therapy. In a study done by Okuyuca, three different shapes of nanoparticles–nanospheres, nanorods, and nanocages–were loaded into their own individual "nanostructured lipid carrier" (NLC) to evaluate their photothermal effect on tumors.[43] The 18.6 nm diameter nanospheres were synthesized via chloroform and CTAB solution, while the nanorods and nanocages were synthesized via seed-mediated growth. This study utilized Type-III or multiple-type NLCs as they contained lipid liquid nano-compartments, which would have allowed for a higher solubility of the drug in the NLC. These NLCs arise as high nanoparticle concentrations are needed to reach the desired hyperthermal temperature; however, high concentrations of gold nanoparticles often accumulate in the main organs. NLCs help with the transportation of these high concentrations to the tumor itself. This study also utilized NIR light for photothermal therapy due to the low attenuation for soft tissues. During the photothermal performance

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of the nanoparticles, it was found for all nanoparticles that as the concentration of the gold nanoparticles in the NLC increased, the temperature change also increased. Gold nanorods were the best photothermal agents as they reached the desired temperature to induce cell death in tumors in less time and concentration. Nanorods also had the highest photothermal conversion efficacy at 30.49%. In other studies, it was examined that the number of tips of gold nanoparticles correlated with its efficacy in photothermal therapy. Yang's team analyzed the effect nanoparticle shape had on photothermal therapy.[41] Like Okuyuca's study, Yang analyzed nanoparticles and nanorods; however, instead of nanocages, Yang analyzed nanostars. After irradiation for 10 minutes, nanospheres had a photothermal conversion efficiency of 21.6%, nanorods had an efficiency of 20.4%, and nanostars had an efficiency of 46.2%, and they triggered the most cell death. It was noted that the high efficiency of nanostars might be due to the multiple tips of nanostars. To confirm that tips affect photothermal efficiency, another study by Saw was examined. Saw's study examined the effect that the size of nanoparticles could have on the impact of photothermal therapy.[45] Confeito-shaped (confeito is a type of spiky candy) nanoparticles were utilized and examined in four sizes: 30, 60, 80, and 100 nm. Before further discussing this study, it should be noted that the existence of tips on nanoparticles creates an increased plasmonic effect, which causes a strong photothermal effect. While 100 nm confeito-shaped gold nanoparticles produced the most number of tips with 75 tips/particle, 30 nm gold nanoparticles had the highest number of tips per unit particle surface area with $8.2x10^{-3}$ *tips/nm*^{2}. Unsurprisingly, the 30 nm nanoparticles led to the highest increase in temperature, with a maximum of about 47 degrees Celsius. As stated earlier in Xia's study, this temperature is perfect for photothermal therapy. The 30 nm nanoparticles also had better cellular internalization and the highest photothermal cytotoxicity effect. This suggests that perhaps the number of tips a nanoparticle has contains a property that induces a high photothermal conversion efficiency through an increased plasmonic effect due to the high density of tips. Of course, to further confirm this, photothermal therapy research with gold nanostars and other nanoparticles shaped with tips would need to continue in this direction to develop this cancer therapy's efficiency further.

The concept of hyperthermia is also crucial to phototherapy. Hyperthermia occurs when nanoparticles exposed to radiation produce heat that raises the temperature of the tumor to 40-45 degrees Celsius.[46] Banu's study investigated the effect plasmonic gold nanospheres, modified with folic acid and loaded with doxorubicin, could have on photothermal therapy using MDA-MB-231 breast cancer line cells. Since these cancer cell lines overexpress the alpha human folate receptor, the folic acid would increase the uptake of gold nanoparticles. The laser exposure time was 30 seconds, and it was found that using these gold nanospheres was very effective in the fatal destruction of cancerous cells. Additionally, it was found that as doxorubicin content increased, the cell viability of the cells decreased. This suggests that combining photothermal therapy and chemotherapy would be the most effective. Roma-Rodrigues' study seems to bolster this argument. The study involved the effect of combination therapy on 3D spheroids of HCT116 colorectal carcinoma cell lines.[47] With the use of 18 nm nanoparticles, widespread cell death at the core of the spheroids was induced through photothermal irradiation. Additionally, the doxorubicin used in the combination therapy was more easily dispersed in the spheroids with the help of these nanoparticles. These results, along with Banu's study, suggest that this combination therapy is an effective method of treating cancerous cells. However, it must be noted that these two studies were *in vitro* studies. To confirm the effectiveness and safety of combination therapy, more *in vivo* studies would need to be conducted as Banu notes that the "future perspective of this research is to extend this approach in animal models and to study the effectiveness of this combination modality of targeted chemotherapy and photother-mal therapy for the reduction of size of tumors".[46]

Of course, many studies have been conducted in which nanoparticles are not necessarily used in combination therapy but are conjugated or tethered to proteins or medicines. Khiavi's team conducted a study in which 18 nm gold nanoparticles conjugated with bovine pancreatic ribonuclease (RNase A) were tested to determine its photothermal capacity in colon cancer cells.[48] NIR laser irradiation was used, and it was found that the gold nanoparticles led to decreased cell viability; however, there was a decreased effective dose of gold nanoparticles on the cell line. Even then, it was noted that this led to significantly eradicating cancer cells. Another study by Yang in which doxorubicin-tethered gold nanoparticles were used to conduct photothermal therapy in mice showed that, with PEG, tumor growth was inhibited.[49] Nanoparticles with a diameter of 38.9 nm were synthesized and used for both the *in vitro* and *in vivo* experiments that occurred in the study. The *in vitro* results found that doxorubicin could be rapidly released during photothermal therapy in murine squamous carcinoma cells with cytotoxicity that depended mainly on the dosage and type of gold nanoparticle treatment. The *in vivo* results found that photothermal efficacy could prohibit tumor growth by using gold nanoparticles. These studies' results are promising, suggesting that the tethering of gold nanoparticles with proteins or medicines could significantly enhance its application when treating tumors.

Drug Delivery with Gold Nanoparticles:

Gold nanoparticles have immense potential in terms of drug delivery. For instance, gold nanoparticles can carry anticancer medicine through fast internalization of active targeting agents, making them more efficient.[50] The usage of gold nanoparticles for drug delivery has been mentioned several times throughout this review, though not thoroughly analyzed. This topic is recurring in many other studies because drug delivery with gold nanoparticles has strong ties with photothermal therapy, cell interactions, and even the considerations of synthesis. For instance, while Okuyuca's study primarily focused on photothermal therapy, it also noted that in NIR irradiation, doxorubicin release increased from 22.4% to 54.7%. The percent release value also increased from 26.6% to 51.2%.[43] Clearly, combination therapy is very much possible; however,

more studies should be published before utilizing combination therapy with gold nanoparticles in clinical trials. Still, these studies show the possibility of gold nanoparticle application for drug delivery.

Khodashenas and her colleagues investigated the effectiveness of different shapes and sizes of gelatin-covered gold nanoparticles in the drug delivery of methotrexate (MTX).[51] 50 nm and 100 nm gold nanospheres and 20, 50, and 100 nm nanorods were investigated. Additionally, the study was performed under pHs 7.4 and 5.4 and temperatures of 37 and 40 degrees Celsius. The study also evaluated the entrapment efficiency and found a direct relationship between size and entrapment efficiency. It was concluded that the gelatin-coated gold nanospheres were much better at drug loading; however, they performed worse at drug release than gold nanorods. In fact, drug release was found to be best with decreasing size (additionally with pH 5.4 and 40 degrees Celsius). This presents a paradox when utilizing nanoparticles suited for entrapment efficiency and drug release rate. Lee's study also examined the effect of pH on the drug delivery system. In this *in vitro* and *in vivo* study, hyaluronic acid-modified dendrimer encapsulating gold nanoparticles (AuDEN) loaded with DOX were utilized to examine their potential in ovarian cancer.[50] These nanoparticles were tiny (1.5 nm) nanospheres that allowed the doxorubicin to bond to the AuDEN through the Au–S bonds. Similar to the study done by Khodashenas, there was a higher drug release rate under the pH conditions of 5.6 rather than 7.4. There was also more cellular uptake with gold nanoparticles when compared to free-DOX. The tumor was significantly reduced in the *in vivo* study in which mice were injected with AuDEN. Additionally, DOX often causes cardiotoxicity; however, due to the active targeting ability of HA, there was a substantial reduction in DOX cardiotoxicity. This could suggest a safer means of DOX delivery. However, there are safety concerns regarding the nanoparticles themselves as they were shown to be extremely small and have a relatively unstable Zeta potential. Another study by Nguyen examined the effect β-cyclodextrin (cyclic polysaccharides made of glucose) functional groups could have on the drug delivery efficiency of 5-FU (a chemotherapy drug).[52] 2-hydroxypropyl-β-cyclodextrin (HPDC) and TMACD were assessed with β-cyclodextrin (CD), and it was found that CD gold nanoparticles and HPDC gold nanoparticles had 13 nm and 16 nm diameters, respectively. In contrast, TMACD gold nanoparticles were characterized as having 105 nm diameters. In accordance with the results found by Khodashenas, the smallest nanoparticles, CD gold nanoparticles, had the greatest drug release at about 29.45%. Unsurprisingly, the loading efficiency was found to be the greatest at the largest nanoparticles, TMACD gold nanoparticles, with an efficiency of roughly 70%. This contrast is significant as it challenges gold nanoparticles in drug delivery systems. Therefore, while these are recent studies, more studies should be conducted to evaluate alternative solutions to this ongoing problem.

Many *in vitro* studies have evaluated nanoparticles' effect on the delivery and toxicity in cancer cell lines. Docetaxel, an anti-cancer drug, was loaded into gold nanoparticles conjugated with folic acid to investigate the effectiveness of lung cancer drug delivery.[53] This study, done by Thambiraj and his colleagues, notes that chemical reduction was used to produce roughly 18 nm nanoparticles. It was determined that these gold nanoparticles significantly caused cell death in the lung cancer cell line (H520). In fact, this study confirmed that increasing the concentration of nanospheres led to even more cell death with 75 μM, causing cell viability to decrease to 39%. In Jawad's study, tumor necrosis factor (TNF-α), a cytokine, was bound to 30 nm nanoparticles to assess the cell death of AMJ13 cells (breast cancer).[54] The results found that TNF-α gold nanoparticles could induce cell death through apoptosis. It was also found that these nanoparticles reduced the Rh123 strain in these cells, which ultimately depleted the mitochondrial membrane potential. This data suggests that these gold nanoparticles, in this study and Thambiraj's study, could be considered for drug delivery.

While this review has focussed mainly on evaluating gold nanoparticles through experimental means, it should be noted that other methods–such as simulations–are used to evaluate the effectiveness of gold nanoparticles. Interestingly, a Monte Carlo simulation was also utilized to produce results about the drug delivery of gold nanoparticles. Asadi's study examined gold nanoparticles' dose enhancement factor (DEF) in choroidal melanoma. It attempted to investigate if normal tissues were affected as severely as the tumor when utilizing gold nanoparticles.[55] It should be noted that the nanoparticles were set to 50 nm as this size is much higher than the other studies discussed. The results showed that gold nanoparticles facilitated a dose increase inside the tumor with no other significant effects in the other parts of the eye. Another Monte Carlo simulation was used to compare dose enhancement factors between regular gold nanoparticles and gold nanoparticles with an iron (II, III) oxide core under a magnetic field. These magnetic gold nanoparticles contained a 60 nm diameter iron oxide core with a 20 nm thickness gold shell.[56] The results showed that in the cell model, higher nanoparticle concentrations led to a higher DEF so long as it was within the sensitive energy range. Additionally, it was found that without a brachytherapy source, the DEF of the magnetic gold nanoparticles was generally lower than the non-magnetic nanoparticles (6% lower in the cytoplasm and 5% lower in the nucleus). However, targeted magnetic gold nanoparticles irradiated by a brachytherapy source showed a DEF 22.17% higher in the cytoplasm and 6.89% higher in the nucleus when compared to the non-magnetic gold nanoparticles. This suggests that, combined with radioactive sources, magnetic gold nanoparticles have the potential to support drug delivery in clinical trials. All the studies analyzed in this section only further the claim that gold nanoparticles have a large potential in drug delivery and chemotherapy.

Gold Nanoparticles in Diagnostic Imaging:

Gold nanoparticles can not only be used for treatment but also detection. Like the other sections, the potential in gold nanoparticle diagnostic imaging is also particularly high. Before continuing with this section, the concept of fluorescence must be discussed. As defined by Miura and Asano, fluores-

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cence results from an emission of light when a fluorophore is exposed to light at its excitation wavelength.[57] This would mean that for the usage of nanoparticles for the detection of cancer, fluorescence would be utilized to evaluate its effectiveness. Usually, in detection, cancer biomarkers would be utilized to determine cancer diagnosis. An example of a biomarker for cancer is the CD44 protein, which is overexpressed in many cancer stem cells.[58,59] A study by Hejazi and her colleagues utilized hyaluronic acid functionalized gold nanoparticles to detect MCF-7 cells while examining incubation temperature and time.[60] These nanorods were sizably large, being about 100 nm in diameter. It was found that incubation temperature decreased the fluorescence intensity due to increased intermolecular interactions, and incubation time increased the internalization amount as red fluorescence emission was detected. It was concluded that the strength of the CD44/HA bond caused the high affinity of gold nanoparticles. Of course, the CD44 protein is not the only cancer biomarker. Shahdeo's study examined gold nanoparticles functionalized with peptides and their imaging of urokinase plasminogen activator receptor (uPAR) cells.[61] In essence, uPAR is a biomarker expressed on many cancer cells like ovarian, breast, and lung cancer. More specifically, growth factor domain (GFD) and somatomedin B domain (SMB) peptides were compared in this study. Peptide was used to functionalize the gold nanoparticles as peptides are stable, less immunogenic, and easily tailored. The gold nanoparticles in this study were coated with chitosan polymer and had a diameter of 20 nm. It was determined that GFD largely contributed to the internalization of gold nanoparticles, but all nanoparticles were able to achieve fluorescence through fluorescein isothiocyanate (FITC). Since the nanoparticles were able to achieve fluorescence through FITC, this study points to the effectiveness of nanoparticles in cancer diagnosis.

Contrast agents could be utilized to distinguish internal structures in computed tomography (CT) scans.[62] These contrast agents achieve a "low contrast resolution detectability", making differentiating different tissues easier. It should be noted that gold nanoparticles are one of many effective contrast agents. Malekzadeh's study focussed on how nanoparticles coated with black iron oxide (Fe_3O_4) could serve as contrast agents in CT imaging. These nanoparticles were 60 nm, as it was noted that nanoparticles smaller than 100 nm could produce more stability and an easier tumor delivery. The nanoparticles in this *in vitro* study interacted with SKBr-3 cells (breast cancer), and it was found that by increasing the cellular uptake time, a stronger fluorescence was achieved. The results suggested that the lower dosage of cells and higher incubation times yielded an increased absorption rate of X-ray photons. The gold nanoparticles enhanced the contrast image significantly enough for imaging centers. However, increasing the time after incubation would decrease the X-ray intensity. While the X-ray intensity would decrease, the contrast image produced still indicates that gold nanoparticles would be extremely useful when differentiating internal tissues, contributing to the detection and diagnosis of cancer.

Background fluorescence intensity is an important issue that must be discussed in cancer diagnosis. In essence, background fluorescence is the fluorescence emitted naturally by molecules, which could interfere with detecting strains or cancer.[63] Xu's study found that gold nanoparticles could be used with DNA-silver nanoclusters (DNA-AgNCs) to quench the background fluorescence intensity.[64] In this study, DNA-AgNCs electrostatically adsorbed onto the gold nanoparticles, resulting in the mentioned quenching of background fluorescence. Then, with the addition of carcinoembryonic antigens (CEAs) and carbohydrate antigen 125 (CA125), the silver nanoclusters are able to bind with the corresponding aptamers. This results in gold nanoparticle aggregation, which leads to fluorescence recovery. This method was tested in the diagnosis for ovarian cancer and was found to be more effective than the monitoring of a single tumor marker. Perhaps different types of nanoparticles can work with each other to further promote the detection of cancer.

In recent studies, gold nanoparticles have been synthesized in greener ways for both safety concerns and biocompatibility. Gold nanoparticles were interestingly synthesized with green tea leaves to be used for biosensing CD44 biomarkers in a study by Ashikbayeva and her colleagues.[58] The nanoparticles (sized between 30 and 50 nm) served as a "transducer part of the biosensor", which showed a high level of binding. The nanoparticles also showed low-limit detection. This concept of green nanoparticles is fundamental when considering safety considerations, as this ties into the next section of this literature review.

Biocompatibility and Safety Considerations:

As seen in this review, the safety and cytotoxicity of gold nanoparticles heavily vary between shapes, sizes, and dosages. There are conflicting results on the safety of gold nanoparticles, so clinical trials are limited. Some safety considerations often include the usage of chemicals in the synthesis of nanoparticles. While many nanoparticles are synthesized with CTAB, chemicals like this can contain byproducts that affect biomolecules.[58] Biocompatibility has become a noticed topic in recent years, and studies like Ashikbayeva's have experimented with "green-synthesized nanoparticles." In Ashikbayeva's analysis, these green-synthesized gold nanoparticles did not affect the viability of the cell, suggesting low cytotoxicity. This remained true at high concentrations. While the concept of these green-synthesized gold nanoparticles remains relatively new, future studies may experiment more with them. In fact, we should highly consider these nanoparticles as they will likely have the highest chance of being approved by the U.S. Food and Drug Administration (FDA).

Currently, the FDA has restricted gold nanoparticles as the characterization of gold nanoparticles for "nanomaterial-specific toxicity screening" has only received partial recognition.[65] Part of the reason nanoparticles are restricted is the formation of the protein corona, which was discussed earlier. This changes the functionality of the nanoparticles as they either aggregate or phagocytosis occurs.[66] This does not mean that the FDA rejects every nanomedicine. The FDA approved On-

tak, Abraxane, Doxil, and many others. Some nanomedicines even date back to being approved in the 1990s. These examples only prove the growing field of nanomedicines. Admittedly, clinical trials are scarce; however, due to the different synthesis methods currently being tested, it is very likely clinical trials will grow within the next decade.

One such clinical trial is examining the diagnostic accuracy of gold nanoparticles in salivary gland tumors.[67] This study started in 2018, and as of 2021, recruitment has been completed. This study examined CD24 as a biomarker in salivary gland cancer and assessed the progression of cancer every three months for 60 participants. While the results of this trial have not been posted, the fact that this clinical trial was approved also showcases the safety nanoparticles have achieved in recent years. Another clinical trial is confirming the safety of a study drug called NU-0129 with a Spherical Nucleic Acid (SNA) platform to help patients with recurrent glioblastoma.[68] This SNA platform includes nucleic acids which are arranged on gold nanospheres, and researchers are using this platform to target the Bcl2L12 gene associated with tumor growth. The study started in 2017, ending recruitment in 2020. There were 8 participants for this clinical trial; all of which had a successful drug infusion and underwent resection. While only one participant had serious adverse effects (though it should be noted that this was not related to NU-0129), all participants had adverse effects to some degree. This clinical trial, coupled with the previously mentioned clinical trial on salivary gland tumors further confirms the potential of gold nanoparticles. Of course, safer and less cytotoxic nanoparticles could be utilized through a green synthesis method; however, these trials still reveal the potential nanoparticles have in terms of biocompatibility. With the emergence of more clinical trials and recognition from the FDA, the confidence in the safety of gold nanoparticles is expected to increase.

Conclusion

This review shows that studying gold nanoparticles is a growing field with much potential. This field has amassed a large amount of popularity, especially in recent years, due to its success in both *in vivo* and *in vitro* trials. More than that, this field is recognized as the future of cancer research. This review focussed on its aspect in cancer research, its various synthesis methods, and its interactions with cells.

In terms of synthesis, it is clear that the "Bottom-Up" method is superior when considering energy preservation. Additionally, it is visibly impossible to determine the "best nanoparticle" as the best nanoparticle differs between different synthesis methods and the type of cancer targeted. This makes combination therapy a hard field of research, as there will never be a perfect nanoparticle equipped to tackle two different purposes. Regardless, smaller nanoparticles usually lead to higher toxicity, suggesting that we should look at nanoparticles with larger sizes in all aspects of cancer research.

Protein adsorption is an important topic when discussing nanoparticle research. This is considered one of the problems of gold nanoparticles that hinder the FDA from approving many nanomedicines. Still, as seen in this review, an increasing number of studies are attempting to reduce the protein corona formation, primarily through the "spikiness" of gold nanoparticles. Bioavailability and cytotoxicity results have shown that gold nanoparticles can achieve high bioavailability or low cytotoxicity. This reveals the bright future for this type of research and its high possibility for FDA approval.

In all of the cancer therapies studied (photothermal, drug delivery, and diagnostic), there seemed to be success in terms of the desired result. Of course, there are still multiple issues that need further research, such as the balance between cell death and necrosis and the efficiency of both drug loading and release; however, perhaps with growing *in vivo* and *in vitro* research, we will soon learn more about the methods to circumvent these issues. Many studies analyzed in this review come from within the last five years. As more evidence is gathered in the upcoming years, perhaps an ideal "solution" for gold nanoparticles in cancer and combination therapy can be found.

The continuing study of green synthesis and applications of gold nanoparticles only further shows that gold nanoparticles have a significant effect on the field of cancer therapy. Perhaps one day, cancer will no longer have a profound impact due to the creation of new nanomedicines.

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■ Authors

My name is Mark Nashi, and I am a current senior at Signal Mountain Middle-High School in Signal Mountain, Tennessee. I will attend Columbia University in the fall, and I am currently planning on majoring in chemistry. I intend to continue with this major for my Ph.D.