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Gold nanoparticles application in liver cancer

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ABSTRACT

Studies have shown that hepatocellular carcinoma (HCC) (the most important type of liver cancer) is the fifth most common cancer, and the third cause of mortality, globally. Although major progress has been made in the treatment and diagnosis of this disease, its eradication remains limited. Consequently, discovering new diagnosis and treatment methods is important. Cancer nanotechnology is an emerging field in medicine with the aim to accomplish advances in both cancer diagnosis and treatment. Gold nanoparticles (GNPs/ AuNPs) have attracted much attention, owing to their biocompatibility (bio-inertness, and low cytotoxicity), ability to chemically modify their surface by attaching multiple types of ligands, and their superior optical properties. This review will focus on the current applications of AuNPs in different aspects, such as imaging, drug and gene delivery, radiotherapy, and photothermal therapy of liver cancer treatment.

1. Introduction

Liver cancer is a major cause of death all over the world [1,2]. Fatality rate of this cancer is very high and approximately all patients die within a year. Most liver cancers are diagnosed during advanced stages, where invasive approach is the only treatment with survival rate of 10–30%. Despite significant advancement in its treatment (surgery, chemotherapy, and radiotherapy), cancerous cell eradication remains limited, and survival rates has not improved substantially [3,4].Therefore, new screening, diagnostic and treatment technologies seem essential. Cancer nanotechnology has emerged as a novel field of medicine with the aim to attain advances in both cancer diagnosis and treatment [5]. Nowadays, nanotechnology has had a serious impact on the development of more effective diagnostic and therapeutic modalities [6]. Due to their unique physicochemical and biological properties, new metal nanoparticles have attracted much interest in nano-oncology field [7]. For example, nanoscale gold spheres are red or violet and silver metal nanoparticles are yellow in aqueous solution. They display a robust UV–vis excitation band, which is not visible in the spectrum of bulk metals, unique and highly tunable optical properties, known as Localized Surface Plasmon Resonance (LSPR). Due to high surface-to-volume ratio of the nanoparticles, their plasmonic intensity is highly sensitive to size, shape and their dielectric nature. These features allow their application for the purpose to develop novel biosensors and chemical sensors [8,9]. A number of metallic nanoparticles have strong magnetic properties, which encourages researchers to use them in a variety of fields, such as biomedicine, magnetic fluids, data storage magnetic resonance imaging (MRI), and environmental remediation [10,11].

Silver, palladium and gold nanoparticles have been shown to have homogeneous and heterogeneous catalytic activity. The shape, size and coating of nanoparticles are the most important parameters in catalytic properties due to their effect on particle interaction and surface-to-volume ratio changes. Metal nanoparticles show altered electronic properties and melt at a much lower temperature in comparison with bulk metal materials. These things occur due to small size and large volume-to-volume ratio of the nanoparticles.

Although the physicochemical properties of metallic nanoparticles

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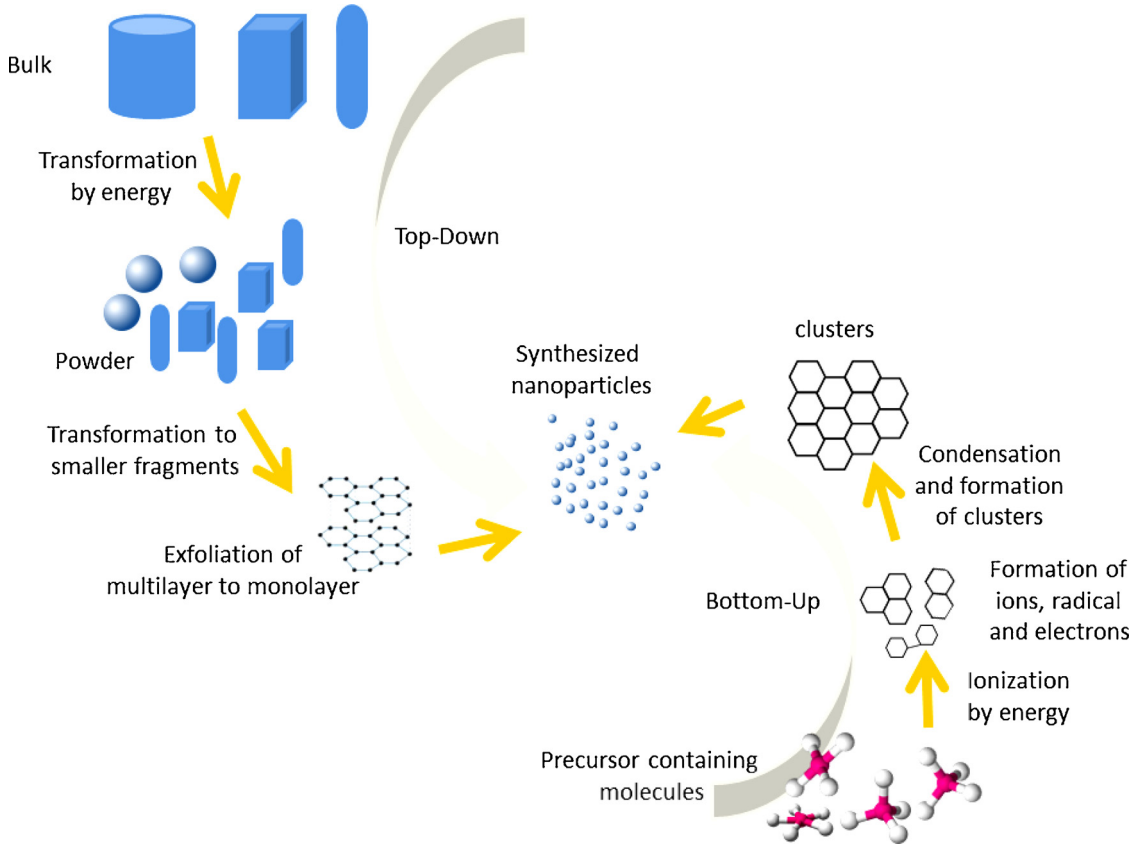
have been well studied, the biological properties of these nanoparticles are still in a state of ambiguity and largely remain unexplored [12]. Today, the antibacterial properties of metallic nanoparticles, such as copper, gold, and silver are well established [13]. Recently, the unique anti-angiogenic property of gold nanoparticles was discovered that led to its nomination in the treatment of some diseases, such as epithelial ovarian cancer and rheumatoid arthritis [14]. Recently it was reported that mangrove-synthesized silver nanoparticles with size ranging from 12 to 28 nm significantly bind to HIV-1 depending on its surface property that inhibit HIV-1 infection [15].

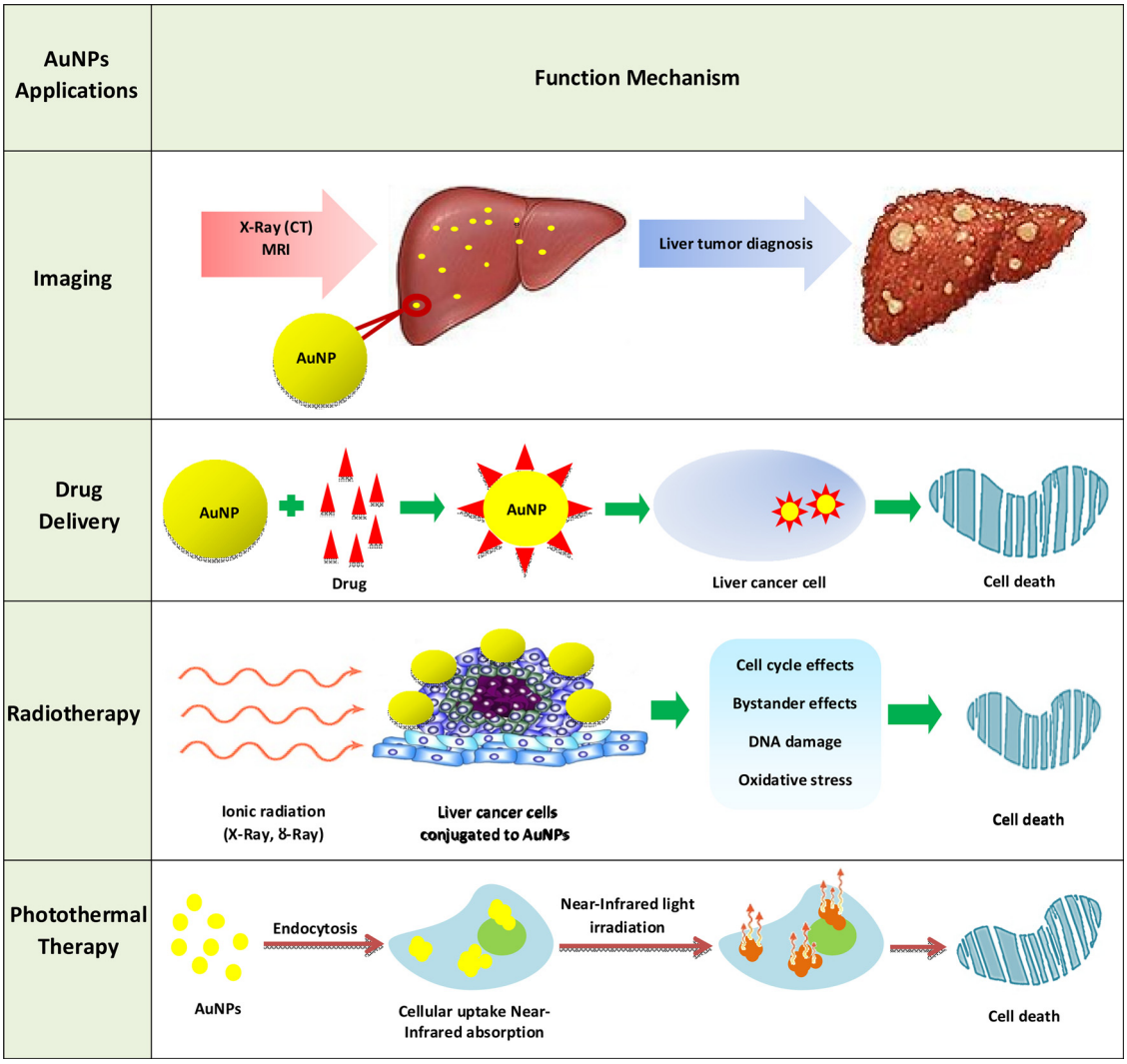
Metal nanoparticles with high surface area-to volume ratios enable simple bioconjugation with therapeutic molecules and/or targeting ligands to a cancer site. They can pass through physiological barriers and passively accumulate within tumor sites due to their enhanced permeability and retention (EPR) effect [16,17]. These NPs can act as agents that simultaneously have therapeutic and diagnostic properties called theranostic. Among these nanoparticles, gold nanoparticles (GNPs or AuNPs) are very attractive for scientists and technologists. GNPs exhibit spherical and non-spherical shapes, such as sub-octahedral, octahedral, decahedral, icosahedral multiple twined, multiple twined, irregular shape, tetrahedral, nanotriangles, nanoprisms, hexagonal platelets, and nanorods [18,19]. Various chemically, physically, and biologically synthetic techniques are applied to produce metal nanoparticles, which are generally divided into two top-down and bottom-up categories (Scheme 1) [20–22].

In the top-down approach, nanostructures are derived by breaking down large-scale materials, using several techniques typically including photo lithography and electron beam lithography, anodization, and ion- and plasma etching (PE). Although these methods are capable of producing pure nanoparticles with specific characteristics, they usually require special and complex equipment which are not cheap and quick to manufacture. Thus, these are not suitable for large scale production. Also, one of the biggest problems with top-down approach is the imperfection surface structure and remarkable crystallographic damage to the processed patterns. In the bottom-up approach, nanostructure is created from smaller building blocks, i.e., metal ions, according to a natural physical principle or an externally applied driving force. Examples for this approach include chemical vapor deposition (CVD), chemical vapor condensation (CVC), thermal decomposition, sol-gel, and aerosol process. Although there are advantages and disadvantages in both approaches, bottom-up approach has more advantage over the top-down approach. The advantages of bottom-up approach are producing nanostructures with less defects and more homogenous chemical composition with the least amount of expenses [23].

Although there are numerous methods for facile production of pure GNPs, the most routine approach is to engage in chemical reduction of a metal salt in the presence of a stabilizer agent [24–26]. In modern medical and biological studies, GNPs are used in different fields due to their unique physicochemical and optical properties. Nowadays, these particles are used in a wide range of applications, such as diagnosis and treatment of various pathologies and diseases [27], wound healing [28], genomics [29], immunoassays [30], optical equipment [31], and electronic [32]. GNPs are strong candidates in cancer nanotechnology because they can be chemically modified by attaching multiple types of ligands, which are biocompatible (bio-inertness, and low cytotoxicity) and have superior optical properties [33]. Most GNPs applications in nanomedicine are based on optical phenomenon, known as localized surface plasmon resonance (LSPR) [34]. When a metal particle is exposed to electromagnetic waves of a specific wavelength, a dielectric can generate a synchronized oscillation in the conduction-band electrons called surface plasmon resonance (SPR) [35]. Typical metal with assignable SPR bands is gold. According to Mie theory, the LSPR band position of the GNPs is dependent on the particle size, shape, structure, composition, and the dielectric constant of the surrounding medium. By changing these factors, GNPs can get tunable absorption in both visible and near infrared (NIR) regions. When LSPR band shift to near infrared



Scheme 1. Nanoparticle formation approaches.



Scheme 2. Different applications of gold nanoparticles in liver cancer.

(NIR) region in the range of 700–900 nm, so-called transparent window, light can penetrate deeply into soft tissues, which make them suitable for in *vivo* medical applications e.g. screening, diagnosis, and treatment of cancerous cells [18,34]. In this review we discussed recent advances in the applications and usage of GNPs for different aspects of liver cancer nanotechnology (scheme 2).

2. AuNPs imaging application

Magnetic resonance imaging (MRI) as a powerful non-invasive technique has a high spatial resolution and ability to distinguish soft tissues [36]. Also, X-ray computed tomography (CT) is one of the greatest useful diagnostic imaging modalities in hospitals, which can provide anatomical information such as shape, location, and size of tissues. Since, soft-tissue contrast is naturally low in CT; hence distinguishing between a special soft tissue from other tissues without using CT contrast agents is difficult. The development of nanoparticles as contrast agents for medical imaging can overcome this problem that include short imaging times, inflammation, difficult to control the size and shape, lack of chemical surface modify, no specificity, and have renal toxicity [37]. Current CT contrast agents are based on iodinated small molecules that have high X-ray absorption coefficient. GNPs are suitable candidates as a CT contrast agent owning to its higher atomic number (^{79}Au versus ^{53}I), large X-ray absorption coefficients, and surface plasmon resonance (SPR) effect [38]. The SPR of AuNPs enhances the electromagnetic fields at their surface and affects the fluorescence properties of fluorophores located nearby. The assembly of AuNPs broadens the SPR band and extends it to the NIR region. Additionally, the space between AuNPs has a strong electromagnetic field, and the fluorescence intensity of fluorophores located near them is tremendously enhanced. Thus, fluorophores located near AuNPs might emit strongly enhanced fluorescence in the therapeutic window, which enable tumor detection using fluorescence imaging and X-ray computed tomography as a dual-mode imaging [39].

AuNPs optical properties make it possible to use them in targeting and detecting materials [40–44]. AuNPs with different ligands can be used as target carriers to deliver drugs or genes to cells in various diagnostic and therapeutic research settings [45–47]. Microorganism-assisted synthesized Au nanoparticles were used to investigate liver cancer cells by conjugating them with liver cancer cell surface-specific antibodies. A study showed that the antibody-conjugated AuNPs bonded specifically to the surface antigens of the cancer cells, could successfully differentiate normal cell populations from cancerous cells [48]. The biodistribution comparison of functionalized Cetuximab with the bifunctional chelating agent pisothiocyanatobenzyl-desferrioxamine moiety labeled with ^{89}Zr (^{89}Zr–Df–Bz–NCS–cetuximab) conjugated and unconjugated to AuNPs were studied by using quantitative PET imaging. Although, liver uptake of AuNPs–plasma-polymerized allylamine (PPAA)–cetuximab–^{89}Zr was higher in comparison with ^{89}Zr–Df–Bz–NCS–cetuximab, there were no significant differences in

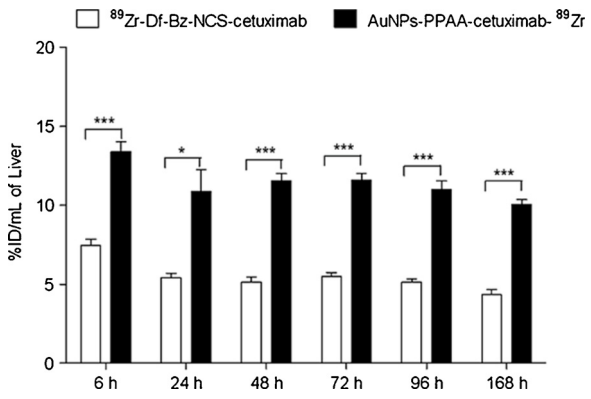


Fig. 1. Liver uptake of ^{89}Zr_Df_Bz_NCS_cetuximab (white bars) or AuNPs-PPAA-cetuximab-^{89}Zr (black bars) in A431 bearing nude mice at 6, 24, 48, 72, 96 and 168 h after injection. Injected activity ranged from 3.3 to 4.6 Megabecquerel) MBq(corresponding to a total of 200–350 μg monoclonal antibody (mAb) per mouse. Results are expressed as means ± SEM (n = 3–5). * $p < 0.05$; *** $p < 0.0001$.

tumor uptake for cetuximab conjugated to nanoparticles up to 72 h after injection in A431 xenograft-bearing mice.

Investigation of immuno-PET studies showed that AuNPs–PPAA–cetuximab– ^{89}Zr provided high tumor-to-background ratio, but did not affect tumor accumulation and the efficacy of EGFR-targeted nanoparticles (Fig. 1) [49]. Generally, a-fetoprotein (AFP), a-fetoprotein variants (AFP-L3) and abnormal prothrombin (APT) are three general tumor markers of liver cancer, identified with three electrochemical redox species with distinct voltammetric peaks. In a study, the electrochemical signals were simultaneously obtained at different peak potentials, and AuNPs coated carbon nanotubes were used to improve the signal response [50]. Also, Gold-silica@4-mercaptobenzoic acid and gold-silica@Nile blue were employed in the surface-enhanced Raman scattering immunosensor for determination of α-fetoprotein and telomerase. The protein biomarkers were taken on the substrate, making a confined plasmonic field, leading to an enhanced electromagnetic field; hence the reported molecules were exposed to a high density of “hot spots” that amplified the raman signal and enhanced sensitivity [51].

SM5-1-conjugated, a humanized mouse monoclonal antibody conjugated to AuNPs, were used for immunotherapy of hepatocellular carcinoma (HCC). Au-SM5-1 NPs showed efficient cell proliferation inhibition and induce cell apoptosis in HCC-LM3-fluce cells in comparison with SM5-1 alone. Moreover, the bioluminescent intensity of SM5-1-conjugated AuNPs was calculated by the developed software Windows Molecular Imaging System (WINMI) to evaluate antitumor efficacy on day 0, 6, 12, 18, 24, and 30 by bioluminescent imaging (BLI) of the tumor-bearing mice. BLI of SM5-1-conjugated gold nanoparticles (Au-SM5-1 NPs) showed tumor accumulation that subsequently inhibits angiogenic in tumor-bearing mice. To reconstruct 3D information of the inner bioluminescence source, BLT and micro-computer tomography technology (CT) were combined. The inhibition rates of Au-SM5-1 NPs was higher than AuIgG NPs, IgG, SM5-1, Au NPs (Fig. 2) [4]. Due to expressing asialoglycoprotein receptors (ASGPR) on $HepG_2$ cells (liver cancer cells), Lactobionic acid-conjugated mercaptosuccinic acid-coated AuNPs can cross cell membrane easily. Hence, they could spcifically recognize liver cancer cells and emit an intense fluorescent signal [52].

Multifunctional nanomaterials make a nanomedical platform that combine individual functions into a single nanostructure that can simultaneously diagnosis and treat. Aggregated gold nanoclusters (A-AuNCs), as a fluorescence source, encapsulated by polyacrylic acid (PAA)/mesoporous silica shell nanoparticles (A-AuNC@-PAA/$mSiO_2$ NPs) show noteworthy aggregation enhanced fluorescence (AEF), which is nearly 4 times more than the discrete AuNPs (Scheme 3). Interestingly, A-Au@-PAA/$mSiO_2$ NPs loaded doxorubicin (DOX) has shown efficient tumor ablation without any systemic damage to the H-22 bearing mice. Also, they have shown the potentiality of dual modal CT and fluorescence imaging as contrast agents (Fig. 3) [53]. Moreover, core-shell-typed mesoporous silica ($mSiO_2$) coated gold nanorods)GNRs-$mSiO_2$(as Janus NPs, showed effective entrance to $HepG_2$ cells to cellular imaging and photothermal effect (Scheme 4) [54]. To reduce the nanoparticle cytotoxicity, polymer layers were coated to increase the surface area and increase the biocompatibility of AuNPs. AuNPs-PEG showed the ability to increase plasma half-life and liver RES escaping [55]. AuNPs functionalized by gadolinium chelates was used as a contrast agent via intravenous injection in mice and rats for both X-ray computed tomography and MRI [56]. Gold nanoparticles coated by Gd-chelate, conjugated to diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid (DTPA) and penicillamine exhibited an ultra-small superparamagnetic iron oxide like behavior, such as contrast enhancement in the liver and lymph node, and the blood pool effect with long circulation time [57].

A Methoxy PEG-iodine-capped gold nanoparticle provides effective enhancement compared to PEG-capped AuNPs with the same amount of gold for X-ray CT imaging. Post-injection of methoxy PEG-iodine-capped AuNPs compared to iodine non-capped gold nanoparticle into the mice showed dramatic contrast enhancement at the heart, aorta, liver, and kidney for 5 day without any apparent toxicity [58]. In addition, AuNP-loaded PEGylated dendrimer showed a blood pool imaging ability, which was greater than a commercial iodine agent. The AuNPs injection into mice displayed liver accumulation with prolonged blood circulation time than iopamidol [59].

In vivo CT imaging of low generation poly (amidoamine) (PAMAM) dendrimer stabilized AuNPs (Au DSNPs) showed approximately the same X-ray attenuation property as that of Omnipaque. The results showed much better performance in CT imaging of major organs of rats *in vivo* than Omnipaque [60]. The lactobionic acid (LA)-modified dendrimer-entrapped gold nanoparticles (LA-Au DENPs) showed *in vitro* and *in vivo* targeted CT imaging of human hepatocellular carcinoma ($HepG_2$ cells) and the xenoplanted tumor model [61]. Au modified amine-terminated generation 5 poly(amidoamine) dendrimers ($G5.NH_2$) with manganese (Mn) chelator, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) fluorescein isothiocyanate (FI), hyaluronic acid HA, (Au°)100$G5.NH_2$-FI-DOTA (Mn)-HA dendrimer NPs, showed favorable characteristics for CT/magnetic resonance dual-mode imaging of HCC-LM3-fluc cells *in vitro* and orthotopically transplanted HCC tumors *in vivo*. The presence of both AuNPs and Mn caused a high X-ray attenuation intensity and favorable r1 relaxivity, which are advantageous properties for targeted CT/MR dual-mode imaging [62]. As it was mentioned, surface modification is important to change some nanaomaterials characteristics. The glycol chitosan (GC) coated AuNPs (GC-AuNPs) showed a tumor targeting CT contrast agent in metastatic liver cancer model [63]. Nanoprobe (M-NPAPF-Au) is a product of co-loading an aggregation-induced emission (AIE) red dye and AuNPs into DSPE-PEG2000 micelles showed long blood circulation half-life, superior tumor-targeting ability, and excellent fluorescence and CT imaging effects in CT_{26} tumor bearing mice (Fig. 4) [64]. Intravenous injection of polyethylenimine-entrapped AuNPs loaded with gadolinium (Gd@Au PENPs) showed only veins distinctly during small dose administration, while both veins and arteries were clearly seen at a larger dose administration with high resolution in mice. MRI showed veins and arteries simultaneously even at a small administration dose, and better resolution with a larger dose [65]. Indocyanine green loaded gold nanorod@liposome core-shell NPs (Au@liposome-ICG) showed to be effectiveness in detecting tumor, and surgery guidance in orthotopic liver cancer mouse models by using photoacoustic and fluorescence dual-modality imaging probe [66].

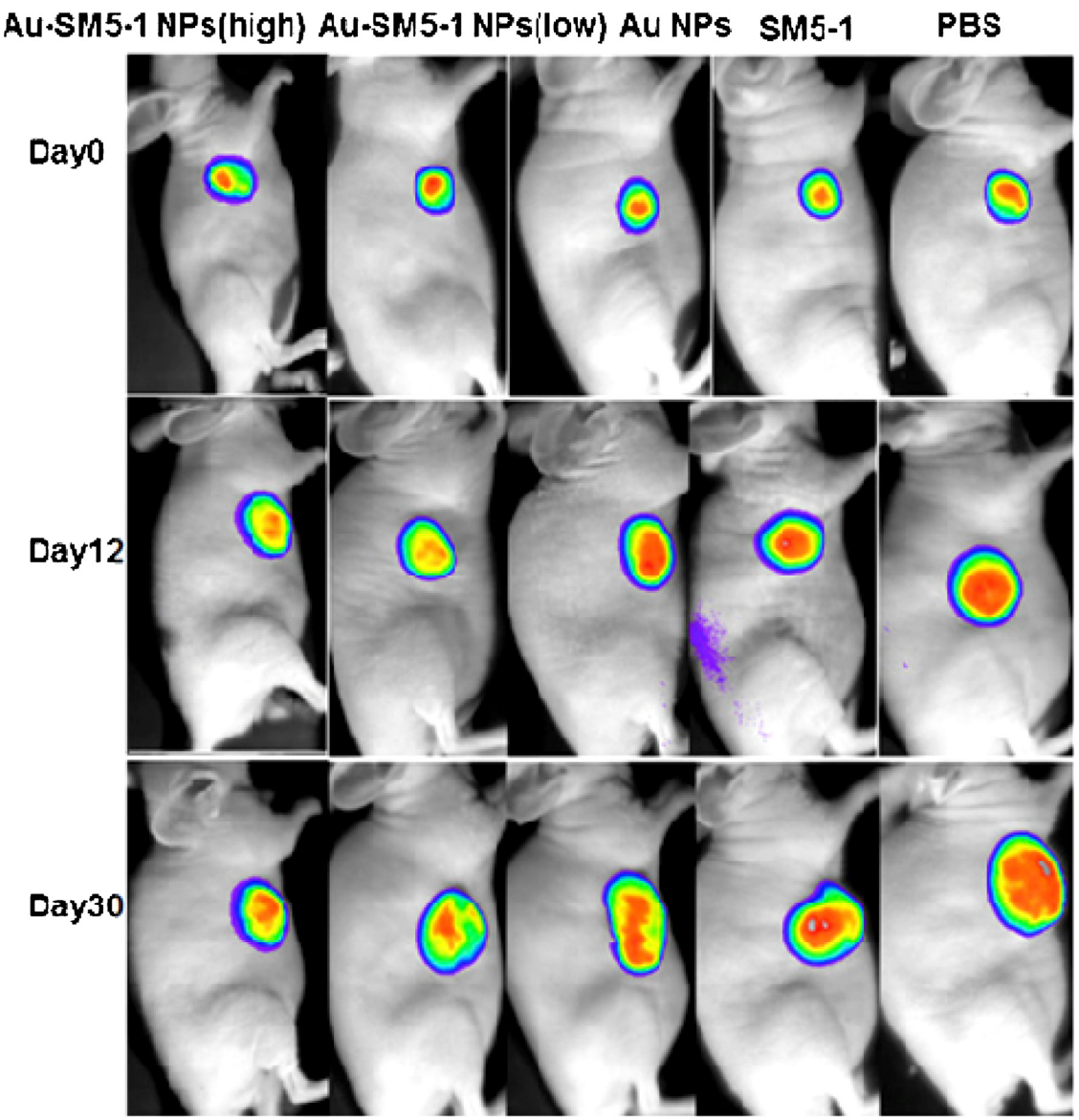


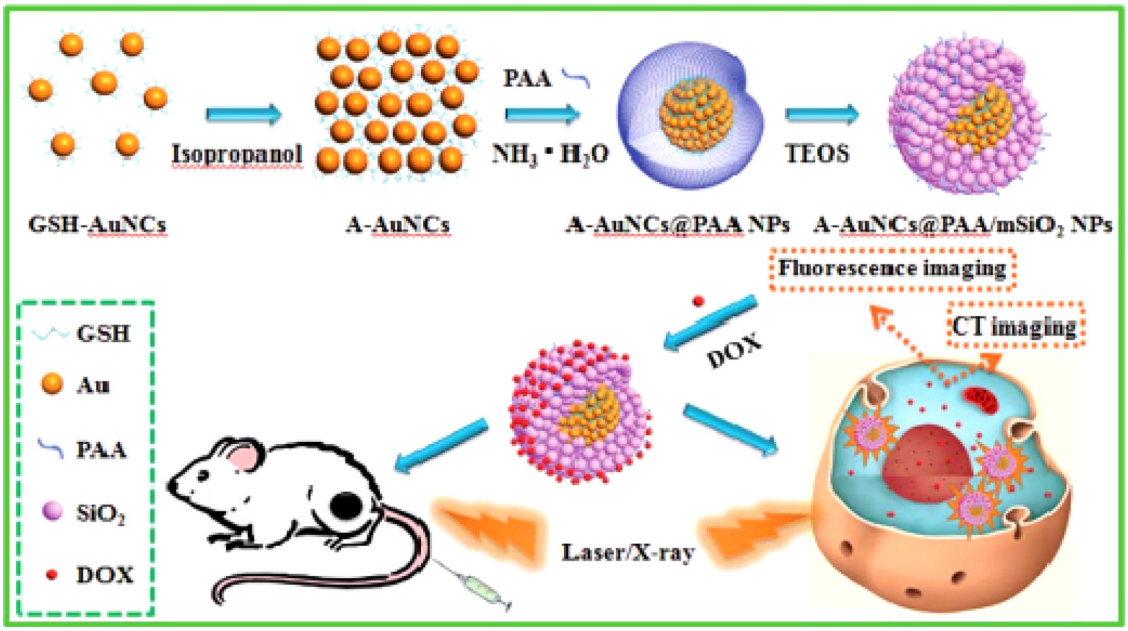
Fig. 2. Serial bioluminescent images of the HCC-LM3-fLuc tumor-bearing nude mice that underwent Au-SM5-1 NPs (high), Au-SM5-1 NPs (low), Au NPs, SM5-1, and PBS treatment at day 0, 12, and 30.

3. AuNPs as drug and gene delivery carriers

Drug delivery is a process to release drugs at a specific location, which reduces the side effects of drugs. AuNP-based chemotherapeutics and gene therapy are widely used for drug delivery and gene delivery. Conjugating GNPs whit drug molecules helps to improve the effectiveness of the drug by adjusting the delivery rate and release to the target tissue. Numerous studies have shown that GNPs have the ability to deliver various molecules, vaccines, recombinant proteins, or nucleotides into their targets. In addition, by modifying AuNPs surface, its properties can be changed for various purposes in cancer therapy.

Pan et al. [67] investigated the effect of GNPs on angiogenesis in hepatic carcinoma-conditioned endothelial cells. Due to its ability to down regulate VEGF activity and disrupt the cellular morphology, they introduced GNPs as antiangiogenic agent in treating hepatic carcinoma. In other words, GNPs can bind to HBD (heparin-binding domain) of VEGF; hence inactive VEGF activity and subsequently inhibit the cell migration and proliferation [67,68].

A study by Tomuleasa et al. [69] showed the use of GNPs coated with L-Aspartate for drug delivery to hepatocellular carcinoma cells (HCC), as a new chemotherapy strategy. They studied three different drugs (doxorubicin, cisplatin and capecitabine) that were non-covalently conjugated with the complex GNPs-L-Aspartate nanostructure, and investigated the effect of these nano-drugs on hepatocellular carcinoma cells [69]. In another study, a hybrid system composed of GNPs and the liposomes were used to evaluate the effectiveness of Paclitaxel (PTX) in liver cancer therapy. For this purpose, two drug delivery systems were examined, one by the hybrid system and the other by GNPs without liposomes. The results of this study showed the efficiency of the hybrid system to be better than the GNPs without liposomes in three properties of solubility, stability and targeting of liver cells [70]. A humanized mouse monoclonal antibody called SM5-1 [71], can be used for target therapy in HCC, due to its ability in targeting an overexpressed membrane protein in HCC, and its potential to induce apoptosis and inhibit cell growth [72–74]. It was shown, when SM5-1 is conjugated with GNPs, it can be used more effectively in HCC therapy in both conditions *in vitro* and *in vivo* [4]. MiR-375 is a tumor suppressor that targets genes through RNA interference. By targeting AEG-1 and ATG7 can suppress HCC malignant traits [75]. In a study, using AuNPs were linked with miR-375 mimic. Xue et al., reported that AuNP-miR-



Scheme 3. Schematic illustration of the synthetic strategy for the aggregated gold nanocluster/polyacrylic acid/mesoporous silica shell nanoparticles (A-AuNC@PAA/mSiO$_2$ NPs) as pH-responsive drug vehicles for simultaneous dual-modal CT and fluorescence imaging and chemo-therapy of liver cancer in vivo and in vitro, (NPs: Nano particles, GSH: Glutathione, PAA: polyacrylic acid, TEOS: Tetraethoxysilane, DOX: Doxorubicin, A-AuNCs: Aggregated gold nanoclusters).

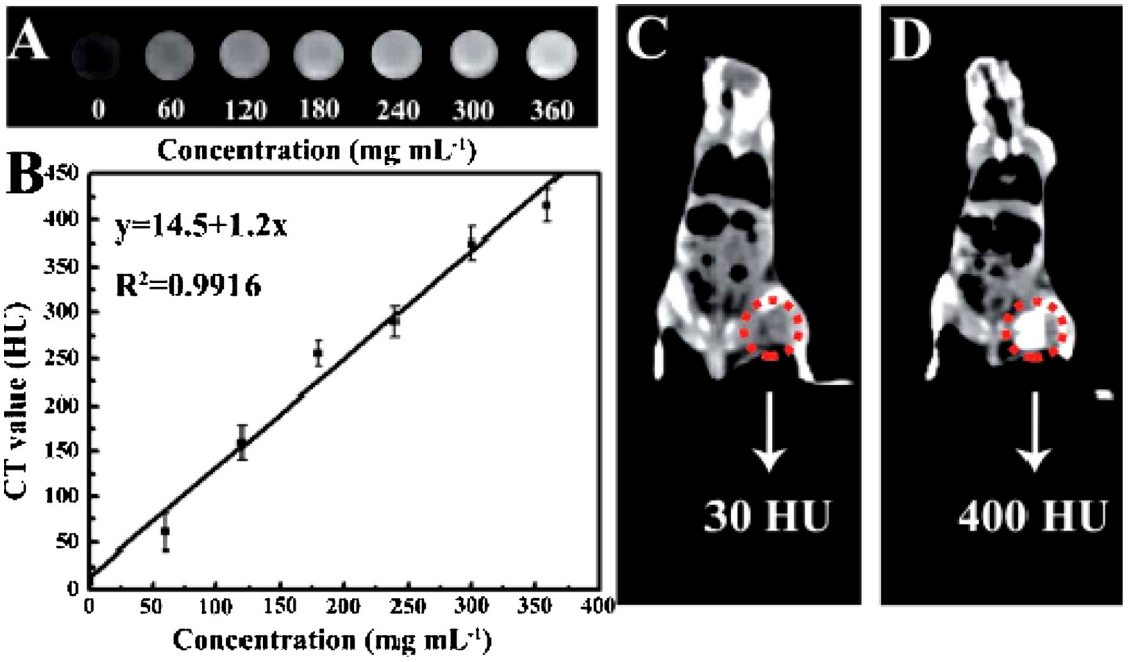
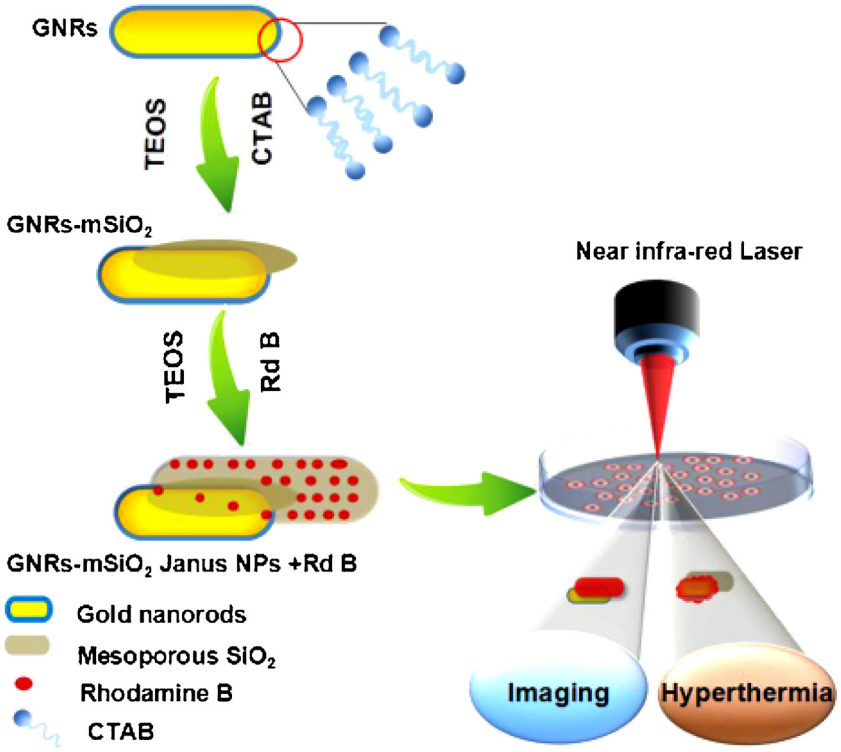


Fig. 3. (A) In vitro CT phantom images of aggregated gold nanocluster/polyacrylic acid/mesoporous silica shell nanoparticles (A-AuNC@PAA/mSiO$_2$ NPs) at various concentrations. (B) HU values of A-AuNC@PAA/mSiO$_2$ NPs at different concentrations. (C and D) are CT phantom images of a tumor bearing Balb/c mouse (C) pre- and (D) post-injection in situ.

375 has the potential to be used for HCC therapy, since AuNP-miR-375 can efficiently deliver miR-375 into HCC cell [76]. Also, miR-122 is specifically expressed in liver cells, and through bcl-w pathway it can induce hepatocyte apoptosis [77]. MiR-122 through simple electrostatic interaction can be conjugated with folic acid-coated gold nanoparticles (G, because of positively charged gold nanoparticles. miR-122 release from the nanoparticles-miR-122-FA nanocomplexes (GMN) and induces apoptosis in HepG2 cancer cells [78]. A study in 2016 showed that GNPs modified by branched polyethyleneimine (bPEI) could be a good option for delivering siRNA to HCC. They designed siRNA against an oncogene c-Myc and then binded it to the bPEI/AuNPs. After HuH7 cells transfection with complex siRNA/bPEI/AuNPs, the expression of the c-Myc by quantitative Real-time PCR (qRT-PCR) was determined. Their results showed that siRNA can effectively silence the c-Myc gene [79].

A research in 2014 showed that GNPs synthesized with *Cajanus cajan* phytochemical [3-butoxy-2-hydroxypropyl 2-(2,4-dihydroxyphenyl) acetate] have the potential to induce apoptosis in liver cancer (HepG2) cells [80]. Also, GNPs produced by thermophilic fungus *Humicola* spp, were used for drug delivery to liver (hepatic) cancer. GNPs



Scheme 4. Synthesis and applications schematic diagram of Janus structure (CTAB: Cetyl trimethylammonium bromide, Rd B: Rhodamine B, TEOS: Tetraethoxysilane, GNRs: Gold nanorods, $mSiO_2$: Mesoporous SiO_2, Janus NPs: Janus nanoparticles).

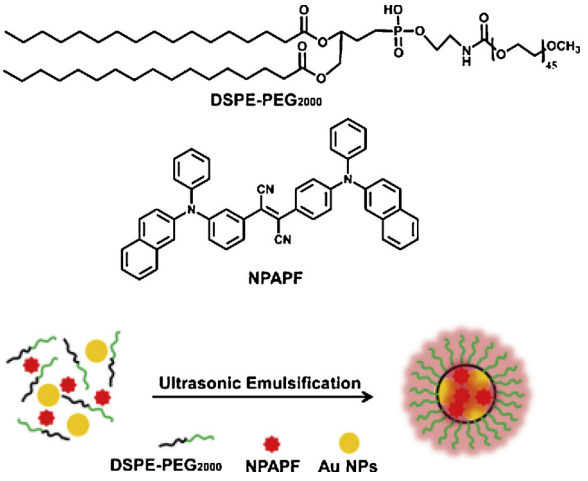


Fig. 4. (a) Molecular structures of DSPE-PEG2000 and NPAPF. (b) Scheme for preparation of M-NPAPF-Au. 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000), Bis(4-(N-(2-naphthyl) phenylamino) phenyl)-fumaronitrile (NPAPF).

synthesized by green synthesis were conjugated with the anticancer drug Doxorubicin that it can be used in targeted drug delivery without the need for any targeting agents like pullulan [81]. In another study published in 2016, the GNPs synthesized by water extracts of the brown seaweed *Sargassum glaucescens* (SG), were able to have an effect on HepG2 cells in *vitro* condition. In the same study, the IC50 values of SG-stabilized AuNPs on HepG2 was reported to be 7.14 ± 1.45 μg/mL [82]. Also, the anticancer effect of GNPs synthesized by marine bacteria Enterococcus sp on HepG2 was investigated. It was found that the synthesized GNPs have anticancer activity potential and its anticancer activity depends on its concentration [83].

Photodynamic therapy (PDT) is a light-based promising non-invasive and unconventional treatment method, which has been a successful clinical track record for the control of a variety of cancers. In PDT, a photo-sensitizing (PS) agent can be applied topically or intravenously and accumulated in cancer cells and then excited with light of specific wavelengths. PDT can destroy cancerous cells by activating reactive oxygen species (ROS). One of the limitations of PS drugs in this methothod is their poor penetration into tissues due to the hydrophobic nature of them. In other words, it is useful for surface tumors treatment [84]. AuNPs have ability to reduce this limit by enhancing the solubility of PS drugs. Also, they can create heat when exposed to appropriate visible or NIR wavelength. Morever, by localizing the surface plasmon resonances, it increases the field of light around the AuNPs, which increases the excitation efficiency in PS [85]. Furthermore, AuNPs is a suitable carrier for PS drugs delivery to target cancer cells. However, Zhang et al., [86] showed that increasing the killing effect of cancer cells by GNPs in PDT is mainly due to its effective drug delivery [87]. Also, the gold nanoparticles alone have the ability to produce singlet oxygen ($^1O_2$) [86]. So far, several studies have been conducted on the effect of AuNPs on the PDT efficacy for liver cancer treatment [88–91]. For example, Chu et al. [90], chose methylene blue (MB or generated ROS) as a model PDT drug molecule and designed three different nanocarrier structures with silica NPs and MB. These designed structures were dense SiO_2-MB, loose SiO_2-MB, and Au@(SiO_2-MB) NPs. They examined the effect of the three designed structures on HepG2. They reported that dense SiO_2-MB NPs suppressed the generation of ROS from the MB trapped in the dense SiO_2 matrix, due to the difficult diffusion of ROS generated in the vicinity of MB. After comparing loos SiO_2-MB NPs with dense SiO_2-MB NPs, they found that loos SiO_2-MB NPs generated more ROS, due to its fragmental nature. However, it generated less singlet oxygen compared to the free MB form. Although, they show that MB trapped in Au@(SiO_2-MB) NPs structure (Au core/silica shell morphology) was able to generate ROS, due to the plasmonic effect of Au NRs and the presence of MB in the vicinity of Au. Eventually, they showed that Au plasmonic effect could increase the drug efficacy by increasing ROS generation [90].

Also Wang et al., [92] encapsulated doxorubicin (DOX) by a

reduction and pH dual sensitive polymeric vesicle and coated its surface with a layer of AuNPs (GNS@PNV) to avoid drug loss in bloodstream. Then, they tested the structures (GNS@PNV) *in vitro* and *in vivo* conditions on Bel-7402 hepatoma cells and nude mice bearing human Bel-7402 hepatoma xenografts, respectively. They showed that after NIR laser exposure to tumor, the GNS@PNV penetrated the tumor tissue and then the DOX was released from it. In other words, NIR light energy on the gold nanoshell caused this layer to break and kill cancer cells by creating heat. Their results showed that photothermo-chemotherapy is an effective method for cancer therapy [92].

4. AuNPs for radiotherapy

Radiation therapy or radiotherapy use ionizing radiation to treat diseases, such as cancer, and less commonly, thyroid disease, blood disorders, and noncancerous growths. The term radiotherapy means the use of high-energy radiation, such as X-rays, gamma rays, electron beams or protons, to treat a disease. This therapy can be curative for some localized cancers or it might be used as an adjuvant therapy to prevent tumor recurrence after surgery. Radiotherapy with chemotherapy has synergistic effect and can be used before, simultaneously and afterwards. It is predicted that about 40 to 60% of cancer patients will benefit from radiotherapy during their treatment. Radiation therapy can be performed both externally/internally. In external radiotherapy, the radiation is delivered by a machine outside the body, but in the internal radiotherapy (also called brachytherapy) radioactive material is placed in the body near the cancerous cells. Radiation therapy has side effects due to its effect on the surrounding area. Most side effects are localized and are usually short-term, while some are systemic and long-term. Short-term side effects include nausea, vomiting, damage to the epithelial surfaces, intestinal discomfort, swelling, infertility, and mouth/throat/stomach soreness, and long-term side effects include fibrosis, epilation, dryness, lymphedema, heart disease, cognitive decline, and even secondary malignancies. Many attempts have been made to preferentially sensitize tumors to radiation and to reduce side effects. There is an effective approach to make the differentiate between tumor and healthy tissues, called therapeutic ratio, using and directing materials with high atomic numbers (Z) into the target tissues. In this regard, iodine, gadolinium and gold nanoparticles with high atomic numbers have been shown to have the ability to radiosensitive tumors. At present, gold nanoparticles are more suitable candidate because of their biocompatibility and meaningful mass energy coefficient relative to soft tissue. The main physical mechanisms of radiation interaction with nanoparticles are Compton and Photoelectric effects, where a photon is absorbed by an atomic electron causing its ejection. In the Photoelectric phenomenon, the ejection of the electron from the inner atomic orbital leads to electron falling out of the layers into outer space, which results in releasing low energy photons, promoting a cascade release of secondary electrons. This phenomenon is called the Auger cascade, leads to the production of low-energy electrons with a range of few micrometers and cause highly localized ionizing events. The main biological responses of cancerous cells to gold nanoparticle radiosensitization includes the production of ROS and oxidative stress, DNA damage induction, cell cycle effects and potential interference with the bystander effects. Physical basis and biological mechanisms of GNP radiosensitization are described in detail by Rosa et al. in the article entitled “Biological mechanisms of gold nanoparticle radiosensitization” [93].

Gold nanoparticles are synthesized by reducing citrate and exposure to RF field in testing solutions or after incubation with HepG2 cells. The nanoparticles were injected directly into the tumors, to concentrate radio waves for selecting heat, and then the temperatures were measured using a thermometer. The results showed that HepG2 liver cancer cells cultured in the presence of GNPs, led to cell death by achieving sufficient heat ($\leq 43\,^{\circ}C$) after being exposed to RF field with no cytotoxicity. Exposing in *vivo* rats at 35 W that received GNPs via direct injection resulted in a significant increase in temperature and thermal injury at subcutaneous areas of the injected sites in comparison with the control group that only received water. According to the results of this study, the radiowave thermal ablation of cancer cells in a non-invasive manner can be facilitated by gold nanoparticles [94].

Nanoparticles in a diameter of 10 nm have been used as a scaffold to synthesis nanoconjugates, producing a targeted antibody (cetuximab, C225) and gemcitabine, which requires 30% efficacy. Combination of targeted gold nanoconjugates with RF was cytotoxic for EGFR, which expressed Hep3B and SNU449 cells compared to isotype particles with/without RF. In addition, this combination led to growth inhibition, increase apoptosis, necrosis, and decrease proliferation in subcutaneous Hep3B xenografts compared to the control. The results showed that combination of radiation-induced hyperthermia with targeted delivery of gemcitabine might be a more effective and safe method in patients with liver cancer in comparison with the control [95].

AuNPs as a radiosensitizer and doxorubicin (DOX) as a model chemotherapeutic were used, and their combination to improve cancer chemoradiotherapy was examined.

A thermosensitive hydrogel based on Pluronic® F127 (Au-DOX-Gel) for loading AuNPs and developed DOX via “cold method” for intratumoral injection was applied and Au-DOX-Gel formulation was optimized at 22% F127 concentration. In *vivo* and in *vitro* release features were compared with the control group, and showed constant release of AuNPs and DOX. Combined treatment of DOX and AuNPs under radiation had inhibitory effects on cell viability and surviving portion in mouse melanoma (B16) and human hepatocellular liver carcinoma (HepG2) cells, and also on tumor cell growth as well as its proliferation. Mice tumor sizes by Au-DOX-Gel were significantly decreased compared to the controls. In *vivo* safety of Au-DOX-Gel was also determined by skin safety tests, histological observations of the organs, and changes in body weight; hence it can be introduced as a promising method to improve chemotherapy [96].

Cancer radiotherapy is related to GNPs ability in cellular uptake, characterized by its surface chemistry and particle size [97–100]. Naked GNPs are unstable and have tendency to aggregate in blood, but nanoparticles coated with polyethylene glycol (PEG) were stable and absorbed by endocytosis [101–103].

Two different average sizes of GNPs coated with PEG which are 14.4 and 30.5 nm in size (size distribution was found generally to be < 20% of the standard deviation) were synthesized. Some parameters including blood stability, cellular uptake, intracellular distribution, cytotoxicity, and radiation therapeutic effect of the PEG-coated GNPs were also checked in two different cell lines (HepG2 and H22 cells). The results showed that PGE-coated GNPs is stable in blood, and can lead to considerable increase in cellular uptake. It was found that the change in surface coating of nanoparticles could result in low cytotoxicity, high sensitivity to radiation in liver cancer, and reduction of two cell lines survival (Fig. 5) [104].

5. AuNPs for photothermal therapy

Photothermal therapy (PTT) is a method that has minimum invasive effects. As the result of the conversion of photon energy into thermal energy, irreversible damage to the cell and ultimately cell death occurs. To apply PTT, the near infrared region (NIR N 700 nm) has better absorption band for the nanoparticle (NP) sensitizers to increase the penetration of light into tissues [105]. The first example of photothermal cancer therapy based on GNPs was reported in 2003 [106]. Recently, GNPs application in photothermal therapy was investigated by many researchers. The photothermal mechanism of GNPs was based on the vibrational effects of these particles, when the surface plasmons resonate under photoexcitation.This localized heat can damage tumor tissues or release of therapeutically important molecules [107,108]. AuNPs carry and strengthen photoactive functional groups to effectively penetrate cancer cells membrane. Since AuNPs have chemically

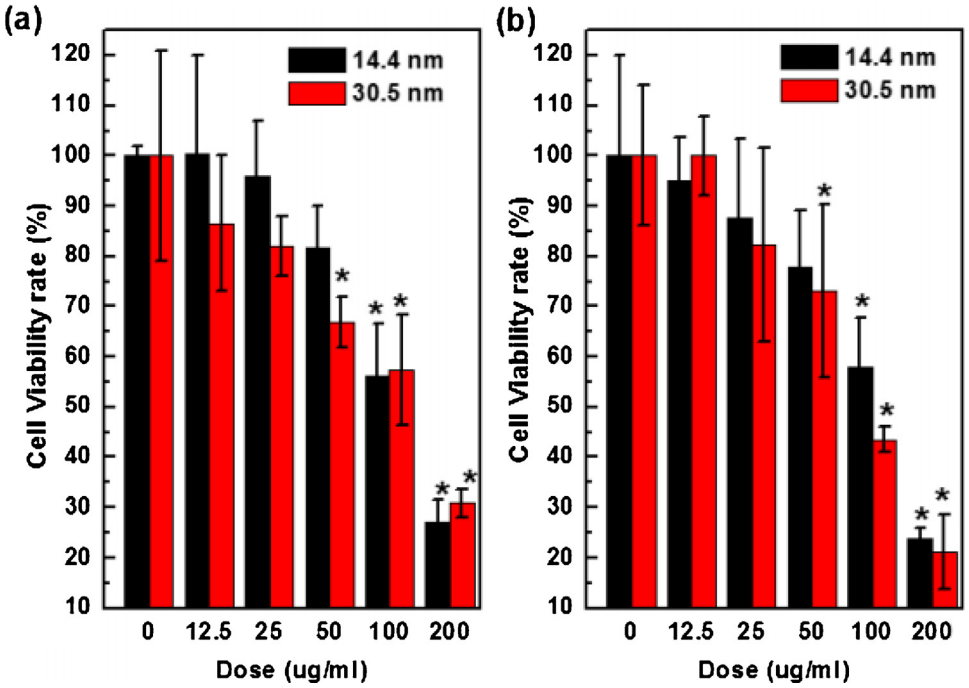


Fig. 5. Concentration dependent toxicity in vitro H22 (a) and HepG2 cells (b) treated with 14.4 or 30.5 nm PEG-coated GNPs after 24 h.

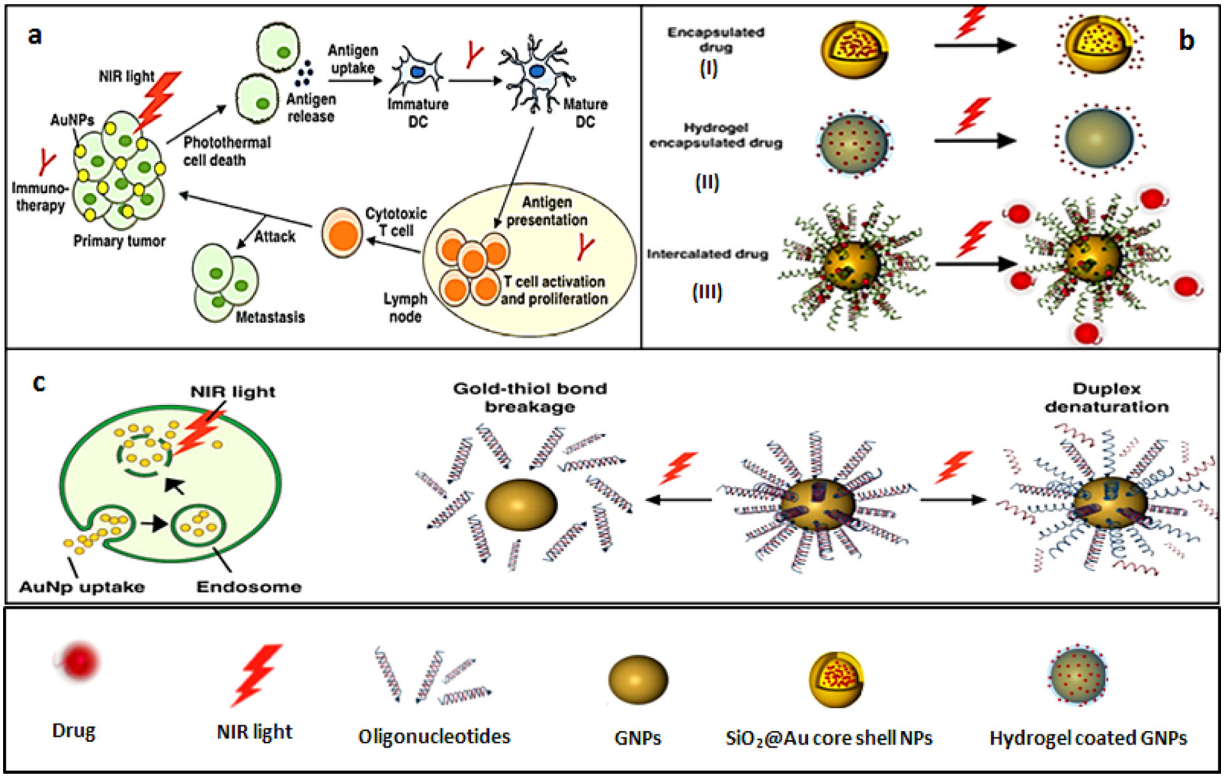


Fig. 6. Combining PTT with other therapies a) impact of combined photothermal therapy and immunotherapy on primary tumors and metastases, b) mechanisms of NIR light induced release of drugs from different forms of AuNPs (I: silica core/gold shell nanoshells and doxorubicin (red), II: gold-nanoshells coated with a thin poly (N-isopropylacrylamide-coacrylamide) hydrogel layer contain drug, and III: AuNPs with oligonucleotides that contain intercalated drugs c) mechanisms of light-triggered gene regulation using AuNPs that gene bonded to AuNPs with thiol groups. (NIR light: Near-infrared light, GNPs: Gold nanoparticles, DC: Dendritic cell).

flexible surface and exclusive photophysical features, they can be conjugated to bioactive components for having effective therapeutic effect on the tumor [108–110]. Conventional hyperthermia and photothermal methods are different from the point that the latter only occurs in the area around the GNPs, which can increase the local temperature up to tens or hundreds of degrees higher than the body temperature in a very short time. This causes the tumor tissues to be targeted by photothermal heating and not the healthy tissues; hence reducing treatments side effects [111]. Although conventional PTT is effective, its usage as a separate treatment is challenging since it might

lead to cell necrosis that increases inflammation and secondary tumor growth. Furthermore, suppression of tumor cells cannot be guaranteed and the use of PTT to treat metastatic disease won't be possible. However, combining PTT with other therapies, such as chemotherapy, gene regulation and immunotherapy can provide a more appropriate treatment (Fig. 6) [112].

Serum albumin (Alb) as a simple GNPs carrier was used to increase laser thermal extirpation of HepG2 cells, and therapeutic effects was shown. To demonstrate the selective internalization of Alb-GNPs into HepG2 cells by targeting the Gp60 receptors, dark field microscopy and immunochemical staining were used. Their results showed that Alb-GNPs led to intracellular uptake increase in liver cancer cells by targeting GP60 receptors selectively, and it was found that after laser irradiation, GNPs photoexcitation resulted in apoptosis by activating caspase-3 [113].

In *vitro* and in *vivo* investigations of Gum Arabic-conjugated gold nanoparticles (GA-AuNPs) and laser combination showed that this method reduces cell viability and activity of histone deacetylase in HepG2 cells. The results indicated that GA–AuNPs, with or without laser radiation can induce apoptosis in cancer cells by activating death receptors (DR5), caspase-3 and also inhibition of preneoplastic lesions (PNLs) prevalence and initial marker (placental glutathione S-transferase; GST-P). In addition, GA-AuNPs stimulation with laser decreased tumor necrosis factor-α (TNFα) levels. Hence, the GA-AuNPs in combination with laser stimulated the extrinsic pathway of apoptosis and inhibited inflammation that can prevent liver PLNs [114].

GNPs conjugated with albumin (BSA) as active vectors were used to target liver cells to develop an alive liver cancer model without any ethical barriers to assess the selective features and remedial capacity of these nanosystems in cancer patients. For this purpose, samples from cancerous patients were perfused out of the body (*ex vivo*). The BSA-GNPs were injected intra-arterially into the specimen, and delivery of the nanoconjugate to the malignant tissue was determined through the capillary bedding. Their results showed that BSA-GNPs accumulation through receptor-mediated endocytosis caused to create a laser-based therapeutic effect at the tumor site, but was not enough to affect the healthy parenchyma tissue around it [115].

6. Conclusion

Amongst the novel metal nanoparticles, GNPs have gained special attention owing to their unique physicochemical and biological properties. This has led to their widespread use of these nanoparticles in the fields of sciences. This review is focused on the use of GNPs in liver cancer, including imaging, drug delivery, and radiotherapy and photothermal therapy. In the case of liver cancer, there are numerous studies about the use of GNPs. The most practical techniques that used GNPs for liver tumor imaging were PET, CT, and MRI. GNPs delivery systems have shown promising results in liver cancer therapy due to their high surface loading ability of drugs and genes.

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