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Applications of gold nanorods in biomedical imaging and related fields

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Gold nanorods (GNRs) have great potential widespread applications in biomedical imaging, drug delivery and photothermal therapy due to unique surface plasmon resonance (SPR) ranging from visible to near infrared (NIR) region, facile synthesis and easy functionalization. In this review, the recent progress of GNRs in bioimaging, drug delivery, photothermal therapy and theranostics is summarized and the recommendations for the future work on GNRs are also covered.

gold nanorods, biomedical imaging, drug delivery, photothermal therapy

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Gold nanorods (GNRs) are rod-like shaped plasmonic nanoparticles with special optical properties because of their anisotropy. GNRs have great potential biomedical applications including biosensing, biomedical imaging, drug delivery and cancer diagnosis and therapy due to their tunable surface plasma resonance, colloidal stability, well-established biocompatibility, easy preparation methods, and easy functionalization. In this article, we review the recent progress of GNRs in the fields of biomedical imaging, drug delivery, diagnostics and photothermal therapy for disease treatment.

1 Synthesis, surface modification and bioconjugation of GNRs

1.1 Optical properties of GNRs

Gold nanostructures have unique surface plasmon resonance (SPR) which results from a collective coherent resonant oscillation with the external oscillating electromagnetic field of the light [1]. The wavelength of SPR is dependent on particle size, shape, structure, the dielectric properties of the metal, and the surrounding medium, as these factors affect the electron charge density on the particle surface [2]. Spherical gold nanoparticles have only one SPR peak in the visible region around 520 nm depending on size and dielectric environment which can be explained by Mie's theory [3]. In contrast, GNRs have two SPR bands, corresponding to a transverse (short axis) and a longitudinal (long axis) band. While the transverse band is insensitive to the size of the GNRs, the longitudinal band is shifted from visible to near-infrared region (NIR) with increasing aspect ratio (length/width). This optical property can be well explained by Gans theory [4]. Due to minimal NIR light absorption by most human tissues and water in the NIR (700–1300 nm), GNRs exhibiting strong NIR absorption and scattering are very attractive for *in vivo* biomedical imaging and drug delivery and therapy applications [5].

1.2 Synthesis of GNRs

A variety of approaches have been developed for the synthesis of GNRs, mainly including electrochemical [6], photochemical [7], template [8] and seed-mediated growth methods [9–11]. Among these, the seed-mediated growth

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method has been the most widely used due to its simplicity, high quality, high yield and ease of particle size controlling. In the seed-mediated approach, GNRs with different aspect ratios can be produced by growing from pre-formed colloidal gold seeds (~1.5–4 nm) in a bulk $HAuCl_2$ growth solution which was obtained by reduction of $HAuCl_4$ using ascorbic acid in the presence of silver ions and cetyltrimethylammonium bromide (CTAB) surfactant. The yield, size and aspect ratio of GNRs can be influenced by many parameters, such as seed concentration, seed size, reductant concentration, temperature, pH, the gold precursor concentration and the surfactant concentration [12].

1.3 Surface modifications of GNRs

GNRs synthesized by seed-mediated growth method have a strong capping of bilayer CTAB surfactant, which provides the surface of nanorods with a positive surface charge and thus ensure their stability in aqueous solution via electrostatic repulsion. However, the CTAB-stabilized GNRs aggregate fast in buffer or cell culture media because of the screen produced by high salt concentration can reduce the repulsive interactions between individual GNRs. Moreover, CTAB-coated GNRs exhibit cytotoxicity to most cells due to the CTAB surfactant itself and the residual or desorbed CTAB [13]. Thus it is necessary to remove or replace CTAB for the advanced utilization of GNRs in various biomedical applications.

The surface modification methods of GNRs include ligand exchange, layer-by-layer (LBL) and surface coating [14,15]. In ligand exchange method, some biological molecules, such as phosphatidylcholine (PC) [16], lipids [17] and thiol compounds, such as alkanethiol [18], thiol-terminated poly (ethylene glycol) (PEG-SH) [19], thiolated chitosan [20], were used to replace CTAB completely or at least partially. The thiol group can form strong Au-S bond with gold surface. The resultant thiol compounds protected GNRs exhibit good stability and reduced cytotoxicity under most circumstances.

CTAB-capped GNRs can also be functionalized by a layer-by-layer (LBL) method in which different charged polymers are sequentially deposited on GNRs surface via electrostatic adsorption. Hauck et al. [21] used LBL technique with two different polyelectrolytes: poly(sodium-4-styrenesulfonate) (PSS) as an anion polyelectrolyte and poly(diallyldimethylammonium chloride) (PDADMAC) as a cationic polyelectrolyte, and Takahashi et al. [22] employed bovine serum albumin (BSA) and polyethyleneimine (PEI) to functionalize GNRs surface to produce the highly stable and remarkably lower cytotoxicity GNRs. However, this approach is tedious and complex compared with ligand exchange method.

Another strategy is to coat GNRs with an inorganic shell (such as silica) [23–26] or a polymer surfactant. Silica is a suitable coating material for GNRs due to its chemical and physical stability, biocompatibility, easy functionalization and further conjugation with biomolecules. Choi et al. [27] encapsulated GNRs into Pluronic-based nano-carriers; resulting in enhanced stability in aqueous solution as well as reduced cytotoxicity compared to that of individual CTAB-capped GNRs. Kim et al. [28] also prepared super-stable GNRS against aggregation under physiological salt conditions by encapsulating in block copolymer micelles.

Although the methods mentioned above can reduce the cytotoxicity of CTAB to some extent, it is still desirable to further develop more easier and efficient approaches to obtain GNRs with high stability and low cytotoxicity.

1.4 Bioconjugation of GNRs

Four methods have been developed for conjugation of biomolecules to GNRs, including direct ligand exchange, covalent coupling, electrostatic adsorption and surface coating. In direct ligand exchange, thiolated biomolecules, such as PEG [29,30] and DNA [31], are directly linked to GNRs by Au-S bonds. In covalent coupling, small bifunctional molecules such as 11-mercaptoundecanoic acids [32] are used as a linker to introduce functional carboxyl groups for further bioconjugation with antibody and protein using carbodiimide coupling agent. Charged proteins, such as antibodies, can adsorb on GNRs via electrostatic interaction. The protein is negatively charged at pH higher than its isoelectric point (pI) and so it can be directly adsorbed to GNRs via electrostatic attraction [33]. This method is easiest; however, its long term stability is still needed to be addressed. Surface coating is using polymeric molecules firstly to coat GNRs with and then the biomolecules react with polymer by electrostatic adsorption or hydrophobic interactions [34]. Compared to the methods of direct ligand exchange and covalent coupling that require ligand exchange, electrostatic adsorption and surface coating are more easier and faster, but the long-time storage stability of the electrostatic adsorption is still uncertain and especially when used for *in vivo* application.

2 Biomedical application of GNRs

2.1 Biomedical imaging of GNRs

GNRs have several remarkable properties, such as strong plasmon absorption band tunable from visible to NIR, enhanced scattering cross section (10^5–10^6 times stronger than that of the emission from an organic fluorescent molecule) and long term photostability. All these features make GNRs very useful for biomedical imaging. The biomedical imaging modes for GNRs include two-photon enhanced luminescence (TPL), dark-field mode microscopy, transmission electron microscopy (TEM), optical coherence tomography (OCT), photoacoustic tomography (PAT), X-ray CT, etc.

Optical imaging is a powerful molecular imaging method and is emerging as a promising technique for the diagnosis

of diseases such as cancer. Among the optical imaging technologies, fluorescence imaging is a desirable modality for early cancer detection due to its low cost, high sensitivity and high spatial resolution. Organic fluorophores and quantum dots are currently used widely as contrast agents for fluorescence imaging applications. However, organic fluorophores are more easily photobleached and quantum dots have long-term toxicity both *in vitro* and *in vivo*. In contrast to organic fluorophores and quantum dots, GNRs have considerable advantages such as biocompatible, no occurrence of photobleaching or decomposition, an enhanced absorption and scattering signal, and tunable longitudinal plasmon absorption from visible to NIR.

GNRs showed strong enhanced two-photon-enhanced luminescence (TPL) due to resonant enhancement of fluorescence emission by the longitudinal LSPR of the rods. TPL has been investigated as a potential technique for cancer diagnosis. The NIR longitudinal band of GNRs overlaps with TPL excitation spectrum, making them ideal probes for TPL-based bioimaging. TPL has the advantages of higher three-dimensional spatial resolution and reduced background of tissue autofluorescence. Several studies have shown the potential of GNRs in cellular and *in vivo* TPL imaging. Durr et al. [35] carried out TPL studies using GNRs in deep tissue and showed that TPL signals of GNRs showed more than three times stronger than those from the two-photon autofluorescence of cells and tissue, making them quite efficient imaging probes for cancer diagnosis. Wang et al. [36] monitored the flow of single GNRs using TPL through mouse ear blood vessels *in vivo* and they found that TPL intensities from single GNRs are many times brighter than the TPL intensities from a single rhodamine molecule.

Due to strong light scattering in NIR region, GNRs can also be used for bioimaging using dark field scattering microscopy. By conjugating specific ligands to GNRs, GNRs have been widely used for cancer imaging with dark filed imaging. For example, Huang et al. [37] used monoclonal anti-epidermal growth factor receptor (anti-EGFR) antibodies conjugated GNRs for distinguishing the cancer cells from the normal cells with a dark-field microscope due to EGFR over-expressed in many malignant epithelial tumor cells. Ding et al. [38] applied dark field light scattering and TEM to image and monitor the transferrin receptor-mediated uptake and biodistribution of GNRs into HeLa cells. The main advantages of GNRs in dark field imaging are that GNRs can be imaged in high-contrast with true color and the multiplexed imaging can be realized using GNRs of different aspect ratios.

Optical coherence tomography (OCT) is another noninvasive scattering-based imaging modality that provides cross-sectional imaging of optically scattering media. OCT uses a short coherence light source to provide three dimensional images of a subject. NIR irradiation allows maximal light penetration into the tissue and image formation is based on the differences in absorption-scattering profiles of the medium. OCT imaging has high spatial resolution in the micrometer range with a penetration depth in the low millimeter range [39]. GNRs have been also used for OCT imaging of cancer. Oldenburg et al. [40] used GNRs for OCT to provide high contrast in an excised sample of human breast invasive ductal carcinoma.

Photoacoustic tomography (PAT) is another new imaging technique developed in recent years. PAT is based on light-induced photoacoustic effects, in which an object absorbs laser pulse light to induce rapid thermal and thus create acoustic waves (ultrasonic emission). The acoustic waves can be detected by ultrasonic transducers to produce images. PAT provides much higher spatial resolution than pure ultrasonic imaging and much higher imaging depth than pure optical imaging. PAT exhibits micron-scale resolution for biological structures compared with the millimeter resolution of ultrasound waves. The imaging depth of PAT can reach several centimeters, while for the pure optical imaging, the depth limits to millimeter scale [41,42]. The amplitude of the photoacoustic signal is directly determined by the absorption efficiency of the object . GNRs are ideal contrast agents for PAT due to the strong surface plasmon absorption in the NIR region where tissue absorption in this region is rather low. GNRs have great potential for both *in vitro* and *in vivo* PAT imaging [43] and have been studied by a few researchers. Eghtedari et al. [44] carried out PAT in nude mice using GNRs and found that GNRs can significantly enhance differences in signal intensity with concentration gradients as low as 1.25 pM. Later, they used antibody-conjugated GNRs for *in vivo* breast cancer PAT imaging [45]. Agarwal et al. [46] demonstrated the ability of PAT for early detection of prostate cancer. Chen et al. [47] found that silica coatings on GNRs can increase the signal to noise ratio of PAT compared with uncoated GNRs.

X-ray computed tomography (CT) is one of the most widely used diagnostic tools in terms of availability, efficiency, and cost. With the appropriate X-ray contrast agent, CT can provide both anatomic and functional information. Compared with small iodinated molecules (current predominant CT contrast agents), which has moderate atomic number and electron density (53 and 4.9 g/cm^3), the atomic number and electron density of gold (79 and 19.32 g/cm^3) are much higher. So gold can induce a strong X-ray attenuation and makes it an ideal candidate for CT contrast agents [48]. von Maltzahn et al. [49] used PEG-functionalized GNRs for X-ray CT imaging and found that GNRs exhibited about two fold amplified X-ray contrast and much longer circulating time (17 h) compared with a clinical iodine standard per mole.

Among all molecular imaging modalities, no single technique is perfect and each has strengths and limitations. For example, optical imaging has advantages of high sensitivity and good spatial resolution. However, it suffers from poor tissue penetration and is highly susceptible to noise due to

the tissue scattering of photons in the visible light. Magnetic resonance imaging (MRI) and computed tomography has good spatial resolution and high imaging depth yet it suffers from low sensitivity. The combination of multiple imaging modalities can offer synergistic advantages over any modality alone. GNRs have also been investigated for multimodal imaging. For example, Luo et al. [50] applied indocyanine green-loaded mesoporous silica-coated GNRs as dual mode imaging contrast agents for both X-ray CT and fluorescence imaging. Sun et al. [51] synthesized multifunctional probes by conjugating Gd (III) ions on GNRs and used them for both magnetic resonance imaging (MRI) and CT imaging. Huang et al. [52] employed multifunctional gold nanorods as contrast agents for both X-ray and photoacoustic imaging. The results showed that this combined modality has the capability to provide anatomical and functional information of tumor for accurate medical diagnosis and imaging-guided therapy. Agarwal et al. [53] developed a dual-modality contrast agent composed of GNRs conjugated to the tumor necrosis factor (TNF-alpha) antibody and is subsequently radiolabeled by ^{125}I for both noninvasive photoacoustic imaging and nuclear imaging in monitoring of antirheumatic drug delivery. This study demonstrates the potential of combining photoacoustic and nuclear imaging modalities through one targeted contrast agent for noninvasive monitoring of drug delivery as well as deep and mineralized tissue imaging. Liu et al. [54] investigated PEGylated GNRs conjugated with a biorecognition molecule, transferrin (Tf) and a magnetic resonance imaging agent gadolinium (Gd) for multimodality imaging. It was shown that Gd incorporation did not interfere with the plasmonic properties of the GNRs and a strong T1 relaxivity was estimated, which is more than twice that of the clinical MRI agent Gd-DTPA. Pancreatic cancer cell overexpressing the transferring receptor served as the *in vitro* model, and the Tf-mediated uptake was demonstrated and confirmed by dark-field imaging and transmission electron microscopy.

2.2 GNRs in gene delivery

GNRs as a nonviral gene delivery vector have been attracted much interest recently [55–57]. Takahashi et al. [58] investigated phosphatidylcholine-modified GNRs for releasing plasmid DNA. After irradiation by a laser light in the NIR at different powers, the shape of GNRs changed from rod to spherical and DNA was released. Chen et al. [59] did a similar research using enhanced green fluorescence protein (EGFP) gene-loaded GNRs for targeting HeLa cells. Wijava et al. [60] loaded and selectively released two different DNA oligonucleotides from two different GNRs by matching laser excitation wavelength to their SPR. This study demonstrated the feasibility of releasing of gene in a remotely controllable way utilizing the optical switch function mediated by GNRs. Masood et al. [61] found that nanocomplex formed by siRNA and GNRs can be used as sensitizers in radiation therapy for head and neck cancer.

2.3 GNRs in photothermal therapy and drug delivery

GNRs are very attractive candidates for photothermal therapy due to their tunable absorption in the NIR region and high optothermal conversion efficiency than most of other shapes of gold nanostructures, such as gold nanospheres and gold nanoshells. Tong et al. [62] investigated the photothermal effects of folate-conjugated GNRs to human malignant nasopharyngeal carcinoma cells and found that the photothermolysis of cancer cells with a high expression of folate receptors was much more effective than that of normal cells with less folate receptors. Norman et al. [63] used antibody-conjugated GNRs to kill Pseudomonas aeruginosa bacterial cells under irradiation with 785 nm light. Dickerson et al. [64] carried out the photothermal therapy of deep-tissue malignancies using PEG-modified GNRs and found that GNRs were preferentially accumulated in tumor sites due to the enhanced permeability and retention (EPR) effect of tumor tissues. The resorption of tumor tissues by laser irradiation was above 57% for the direct injection of GNRs and about 25% for the intravenous injection of GNRs.In comparison, no tumor resorption was detected for all controls. Choi et al. [27] used GNRs encapsulated in chitosan-modified Pluronic for tumor targeting and photothermal therapy. They found that GNRs showed over 20% accumulation in tumor tissues and the tumors were completely resorpted within 6 days after two times of laser irradiation. Li et al. [65] also investigated RGD peptide conjugated GNRs for *in vivo* photothermal therapy. The results showed that the tumor completely disappeared after four times irradiation within one month.

Apart from photothermal therapy, GNRs are also a good candidate for drug delivery and the photothermal effect can be employed to actively release drugs. Alkilany et al. [66] utilized the hydrophobic region near the GNRs surface provided by CTAB bilayer to sequester a model drug-1-naphthol. Alper et al. [67] studied the controlled release behavior of fluorescence dye loaded GNRs under laser irradiation. Wei et al. [68] investigated thermo-sensitive polymer coated GNRs as a drug delivery carrier. The heat generated by irradiating GNRs with NIR light induced a phase transition of polymer and thus released the drug. Hauck et al. [69] found that the heat produced by GNRs can increase the efficacy of chemotherapeutic agents and therapy using drug-loaded GNRs can kill more 78% cancer cells than only drugs treated process. Agarwal et al. [70] designed a thermosensitive liposome loaded with PEGylated GNRs and doxorubicin. When stimulated using near-infrared light, remote triggered release of doxorubicin from thermosensitive liposomes was achieved in a mouse tumor model of human glioblastoma (U87), resulting in a significant increase in efficacy when compared to nontriggered or nonthermosensitive PEGylated liposomes.

2.4 GNRs in theranostics

Theranostic nanoparticles, which combine both therapeutic and diagnostic capabilities in one dose, have great promising in disease treatment [71,72]. GNRs have been developed as theranostic nanoparticles in several studies. Huang et al. [73] demonstrated *in vitro* that GNRs can be used for both molecular imaging and photothermal therapy. They used anti-EGFR antibody-conjugated GNRs to specifically label cancer cells. Due to the strongly scattered red light from GNRs, the cancer cells are clearly visualized and distinguished from the normal cells under dark field mode microscopy. Following laser irradiation, malignant cells were killed at about half of the laser influence needed to kill the nonmalignant cells. Huang et al. [74] carried out *in vitro* dark field mode microscopy imaging, photothermal therapy and *in vivo* X-ray CT imaging using folate-conjugated GNRs. Zhang et al. [75] developed mesoporous silica-coated GNRs as a novel cancer theranostic platform. The inner GNRs core functioned both as imaging agent and hyperthermia agent. The outer mesoporous silica shell allowed a high chemotherapy drug (doxorubicin hydrochloride, DOX) payload, thus posing itself as an effective drug carrier. Two-photon imaging (TPI) was employed to image the intracellular colocalization of DOX-loaded GNRs and some cellular compartments. NIR laser irradiation at a low intensity is used to enhance drug release from carrier for chemotherapy, while at higher irradiation intensity it also triggers hyperthermia of the AuNRs. Wang et al. [76] demonstrated a novel multifunctional nano-pearl-necklace based on Fe_3O_4 nanoparticles decorating GNRs that can be used for MRI, fluorescence imaging and photothermal therapy. They found that these multifunctional nanoprobes exhibited a remarkably stronger MRI contrast than free Fe_3O_4 nanoparticles. After only 5 min irradiation at 785 nm using NIR laser, the breast cancer cells have been killed. Choi et al. [77] used cetuximab (CET)-conjugated PEGylated gold nanorods (CET-PGNRs) as smart theragnostic nanoprobes for image-guided cancer therapy. CET-PGNRs exhibit excellent tumor targeting ability and strong potential for simultaneous absorption imaging and photothermal ablation of epithelial cancer cells.

3 Conclusion

GNRs have shown great potential applications in biomedical diagnosis and therapeutic treatments as a result of their exceptional optical, physical and chemical properties. Gold nanoparticles-based nanoplatforms are still at the initial stage of development and much more research is required before their real clinical applications. The main issues to be addressed involve toxicity, biodistribution, long-term stability under biological environment and the final fate of the GNRs after *in vivo* administration. Moreover, the development of GNRs-based multifunctional theranostic platforms combining diagnostics and treatment are the research direction in the future.

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