

Stimuli-Responsive Systems of Therapeutics

Review

Gold Nanorods as Nanodevices for Bioimaging, Photothermal Therapeutics, and Drug Delivery

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Gold nanorods are promising metals in several biomedical applications such as bioimaging, thermal therapy, and drug delivery. Gold nanorods have strong absorption bands in near-infrared (NIR) light region and show photothermal effects. Since NIR light can penetrate deeply into tissues, their unique optical, chemical, and biological properties have attracted considerable clinical interest. Gold nanorods are expected to act not only as on-demand thermal converters for photothermal therapy but also as mediators of a controlled drug-release system responding to light irradiation. In this review, we discuss current progress using gold nanorods as bioimaging platform, phototherapeutic agents, and drug delivery vehicles.

Key words gold nanorod; bioimaging; photothermal effect; therapeutics; drug delivery

1. Introduction

Gold nanoparticles are highly promising metals in biomedical applications because of their unique optical, chemical, and biological properties. The importance of gold nanoparticles in biomedical applications derives from their tunability of optical properties depending on their sizes and shapes. Because of the capability of gold nanoparticles to bind a wide range of organic molecules, their low cytotoxicity, and their strong and tunable optical absorption, the combination of biological molecules and pharmaceutical products with gold nanoparticles is becoming an increasingly popular theme in biomedical fields. Moreover, gold nanoparticles have played an important role as drug and vaccine carriers into target cells or specific tissues.^{1,2)}

Many different types of nanoparticles have emerged as drug carriers, including organic (e.g. polymers and liposomes), inorganic (e.g. magnetite nanoparticles, various quantum dots, silver and gold nanoparticles), and protein-based examples (e.g. viruses and albumin). Depending on their size and shape, gold-based nanoparticles have additionally unique optical properties for the interaction with light such as strong absorption and scattering in the visible–near-infrared (NIR) region. These nanoparticles have both EPR effects¹⁾ and high surface area to act as nanocarriers.^{3–11)} The large extinction tuned in NIR region is also useful for gold nanoparticles to exhibit efficient photothermal conversion for applications in photothermal therapy. As for non-invasive optical imaging and therapeutic sensing, light with wavelength 750–1300 nm is useful because its absorbance by water and hemoglobin are at minimum, providing a so-called therapeutic window for living bodies.

Gold is also one of the most chemically inert metals and has a high electron density. These properties support gold nanoparticles as good candidates for bioimaging agents. Be-

cause of these properties, it is possible to locate them in complex environments such as in tissues. In this review, we discuss an overview of bioimaging, therapeutic application, and controlled delivery of biofunctional molecules using a type of gold nanoparticle known as gold nanorods.

2. Preparation of Gold Nanorods and Their Optical Properties

Gold nanorods are rod-shaped anisotropic gold nanoparticles that are commonly prepared in micellar solution of cationic detergents. They can be obtained by different kinds of reproducible and controllable methods such as electrochemical synthesis, wet chemical seed-mediated synthesis, lithographic fabrication, template-assisted synthesis, and UV photochemical reduction of gold salts.^{5,12,13)} Yu *et al.*,¹⁴⁾ in 1997, reported the production of gold nanorods in a micellar solution of hexadecyltrimethylammonium bromide (CTAB) using two-electrode electrolysis. That was the first preparation of uniform gold nanorods using a “soft template.” Jana *et al.*^{15–17)} similarly reported seed-mediated gold nanorod synthesis using a suitable reaction solution. The seed particles were prepared in a CTAB solution using NaBH₄ as a reducing agent. Rapid reduction by NaBH₄ yielded small gold nanoparticles approximately 2 nm in size. CTAB enhances the growth of gold seeds to form rod-shaped nanoparticles.^{4,5)} For these prepared gold nanorods, their surfaces were coated with a positively charged CTAB bilayer. Consequently, this avoids aggregation of gold nanorods in water *via* electrostatic repulsion. Using the seed-mediated method, gold nanorods can be produced from circa 1.5–4-nm seeded-gold colloid with predictable size and shape. This has been successfully performed using a low amount of additive silver ions. In the presence of silver ions, gold

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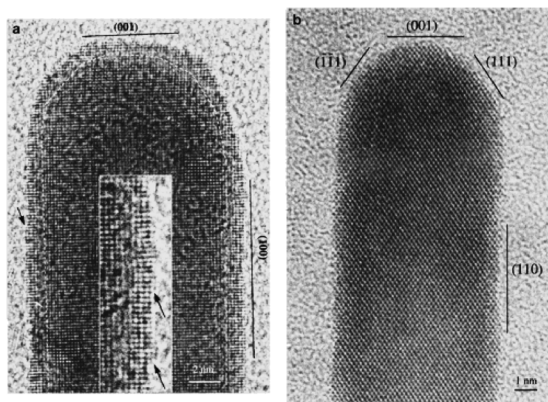


Fig. 1. High-Resolution TEM Image Showing the Facets of a Single Crystal Nanorod

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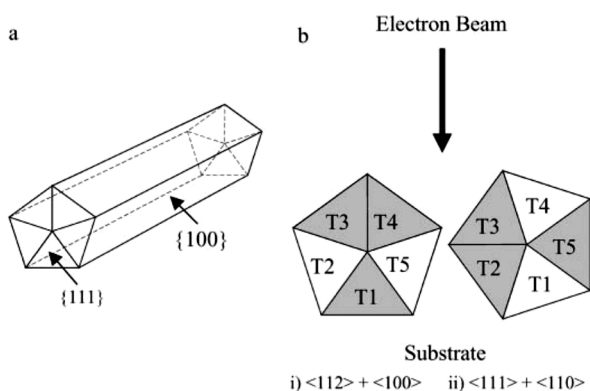


Fig. 2. Schematic Illustration of Penta-Tetrahedral Twin Model of Gold Nanorods

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nanorods form single crystals with side facets of $\{110\}$ and $\{100\}$ ¹⁸⁾ (Fig. 1). In the absence of silver ions, gold nanorods are formed as five-fold twinning crystals with side facets of $\{100\}$ ¹⁹⁾ (Fig. 2). Therefore silver ions are shape-regulating reagents for gold nanorod formation in CTAB solutions.²⁰⁾ Although addition of silver ions changes the crystal structures of gold nanorods, this application does not affect their transverse diameters.^{20,21)} Further detailed information for the synthesis of gold nanorods by chemical reaction is available in several review papers.^{4,5,22,23)} Photochemical method is an alternative means to synthesize uniform gold nanorods using the same reaction solution with that of the seeding method.^{24,25)} In this method, UV light irradiation triggers radical production in the reaction solution. This is an important point to obtain uniform gold nanorods.²⁶⁾ According to these reports, the uniformity of gold nanorods synthesized by photochemical method was improved in comparison with chemical seeding methods.^{27–29)} Detailed mechanisms of formation of gold nanorods have been published in a number of reports.^{30,31)}

The coating layer of gold nanorods, comprising CTAB, is cytotoxic; therefore it is important to modify or functionalize this layer for use in biological purposes. For modification of gold nanorods, many different methods can be applied either to partially remove or overcoat the CTAB bilayer. Layer-by-layer polyelectrolyte deposition is an excellent technique in

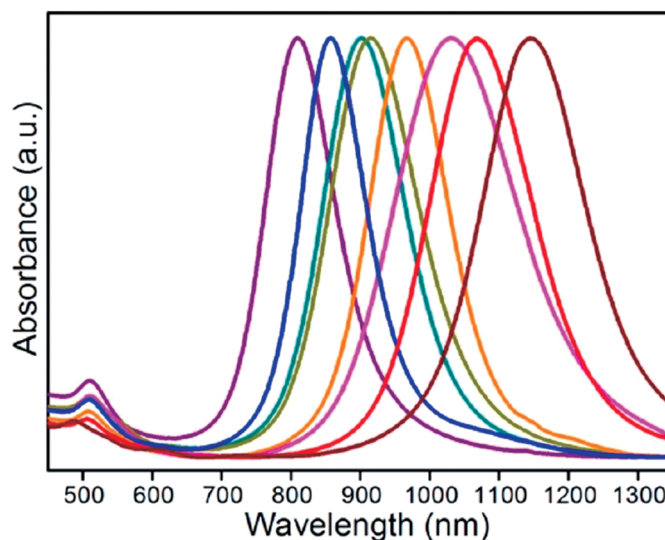


Fig. 3. Normalized Absorption Spectra of Gold Nanorods Having Different Aspect Ratios

As the aspect ratio increases, the corresponding surface Plasmon (SP) band shifts toward longer wavelengths. Reprinted with permission from Wu *et al.*²⁹⁾

which sequential adsorption of anions followed by addition of cationic polyelectrolytes is performed on the surface of gold nanorods.³²⁾ This method allows for modification of both cationic and anionic gold nanorods by means of attaching the targeting molecules.^{33,34)} Other “replacement” reactions may include thiol groups to gold nanorods.^{35–39)} Moreover, gold nanorods can be modified with a silica shell following attachment of desired molecules.

Optical properties of gold nanorods have been reported by Edgar *et al.*³⁸⁾ Gold nanorods showed two surface plasmon bands in visible and NIR regions²⁹⁾ (Fig. 3). The band in the NIR region was the longitudinal surface Plasmon (SP) band that originated from collective motion of free electrons in the gold nanorods. Ellipsoidal approximation of the gold nanorods could predict the peak positions on the aspect ratios (longitude/transverse) of the gold nanorods.^{38,39)} The absorption band in the visible region corresponds to the transverse mode of SP oscillation.

3. Gold Nanorods for Bioimaging Techniques

Many bioimaging techniques have been reported using the unique optical properties of gold nanorods. Gold nanorods have an extinction band originating from light scattering and absorption at the NIR region. First, Huang *et al.*⁴⁰⁾ observed cultivated cells taking up gold nanorods modified with anti-epidermal growth factor receptor (EGFR) antibodies for specific binding under dark-field microscope. Light scattering from the gold nanorods was detected in the cells. Another imaging technique, optical coherence tomography (OCT), is an important bioimaging method with micrometer resolution and three-dimensional (3D) morphology. The underlying principal involves coherence-gated detection of scattered NIR light. In 2011, Jung *et al.*⁴¹⁾ performed OCT bioimaging of gold nanorods uptake in sentinel lymph node of mice. X-Ray CT is a powerful imaging technique with high resolution. Since gold nanorods have stronger absorption than contrast agent, the distribution of gold nanorods can be observed by X-ray CT.⁴²⁾

Bioimaging is one of the most striking applications for two-

photon luminescence because it gives visible emission following NIR irradiation whose excitation enables users to minimize autofluorescence and scattering of biological samples.⁴³⁾ Imura *et al.*⁴⁴⁾ showed scanning near-field optical microscopic images of gold nanorods. Cellular uptake of gold nanorods was visualized by two-photon luminescence microscope.^{45,46)} To detect two-photon luminescence, femtosecond pulsed laser light is required to observe the uptake; however, it can be expanded to direct imaging of single gold nanorods *in vivo*.⁴⁷⁾

Photoacoustic imaging is a well-known imaging technique for non-invasive imaging of deep tissues, in which gold nanorods act as contrast agent. Absorption of pulsed laser provides acoustic shockwaves and signals due to transient heating and thermo-elastic expansion. These signals are converted into an image by scanning transducer. Eghtedari *et al.*,⁴⁸⁾ in 2007, could visualize biodistribution of gold nanorods in nude mice after subcutaneous injection.

4. Gold Nanorods for Therapeutic Applications

The chemical reactivity of even very small gold nanoparticles is important because it can cause oxidative damage to cells.^{49–51)} Indeed, it can be considered that gold nanoparticles themselves might be “drugs” in sufficiently high doses. Photothermal therapy with functionalized gold nanorods has been performed on cancerous and bacterial cells using *in vitro* and *in vivo* models.^{52–54)} Photothermal therapy is a minimally invasive treatment method to induce hyperthermia of tumor cells. Gold nanorods have been also used as “theranostic” agents in photothermal therapy; this is one of the obvious goals of gold nanorod applications in biomedical field. As discussed above, gold nanorods have stronger and more tunable absorption band at NIR light compared with other gold nanoparticles; therefore it is expected that irradiation to gold nanorods can support sufficient heat for thermal therapy. Significant suppression and thermal ablation of tumor growth were observed after irradiation of direct and intravenously injected PEG-modified gold nanorods to tumors in mice.^{55–58)}

Huang *et al.*⁵⁴⁾ reported their experiments using gold nanorods as photothermal therapeutic agents to kill cancer cells. They conjugated gold nanorods with monoclonal antibodies recognizing proteins that are overexpressed in cancer cell lines. For comparison, the gold nanorods were incubated with a non-malignant epithelial cell line as well as malignant epithelial cell lines. As expected, the antibody-conjugated gold nanorods were found binding to the malignant cell lines. After irradiation with a continuous 800 nm laser, cell death resulted even at low laser powers of 80 mW. In more work, von Maltzahn *et al.*⁵³⁾ revealed that functionalized gold nanorods cause photothermal ablation of tumors *in vivo* after a single dose. They injected polyethylene glycol (PEG)-modified gold nanorods into nude mice with xenograft tumors. The tumors were then irradiated with an 810-nm laser resulting in temperature increases to 70°C after 5 min. This therapeutic irradiation regimen was sufficient fully to destroy all irradiated tumors and there was no tumor re-growth for at least 50d until the mice were sacrificed.⁵³⁾

5. Gold Nanorods for Drug Delivery Systems

Cancer is the primary target in nanoparticle-based controlled release system.^{59–61)} For metallic nanoparticles such as gold nanorods that can support photothermal effect, the heat

converted from absorbed light can be applied actively to release drugs. Hence gold nanorods have been employed as drug delivery vehicles *in vitro*,⁶²⁾ although they have been rarely assessed for *in vivo* delivery.

Gold nanorods can be used in photothermal nanodevices for the release of genes and biomolecules. Yamashita *et al.*⁶¹⁾ in 2011, modified PEG-linked Diels–Alder cycloadducts on gold nanorods so as to create a controlled-release system triggered by retro Diels–Alder reaction induced by photothermal effect of the gold nanorods. Diels–Alder reaction is generally known as a reversible cycloaddition reaction between diene and alkene groups to form a cycloadduct. After laser irradiation to gold nanorods or heat treatment, PEG chains were released from the gold nanorod surface because of the retro Diels–Alder reaction. If a modified drug was attached on the PEG chain, the controlled-release system of a drug will be successfully established.

In controlled release means, gold nanorods can be coated with a thermo-sensitive shell in which drug molecules are dispersed. NIR irradiation of gold nanorods generates heat, which induces a phase transition to the expanded form and thus releases the drug.⁶³⁾ Another example is polymer-coated gold nanorods act as a drug reservoir with minimal premature release. In 2013, Shen *et al.*⁶⁴⁾ modified DOX-loaded gold nanorods (GNR-PCL-*b*-PEG-DOX) that could also be used for the treatment of tumors. This research showed synergistic contribution of chemotherapy and phototherapy.⁶⁴⁾ They formulated the DOX-loaded gold nanorods with mesoporous silica-encapsulated gold nanoparticles modified with folic acid (MSNPs-FA) nanoparticles as synergetic effect for localized heating and chemotherapeutics to targeted tumors, in which 1-tetradecanol (TD) was used as phase-changing molecule and gatekeeper to control drug release.

6. Conclusion

As highlighted in this review, there is significant interest in gold nanorods for bioimaging, therapeutics, and drug delivery applications. Their biologically relevant sizes, ability easily to functionalize with biomolecules and chemotherapeutic agents, and their enhanced optical properties suggest they are promising agents for therapeutic drug delivery and noninvasive disease diagnostics. In sum, gold nanorods are incredibly useful as nanodevices for next-generation biomedical applications.

Conflict of Interest The authors declare no conflict of interest.

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