Recent Advances in Hollow Gold Nanostructures for Biomedical Applications

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The localized surface plasmon resonance of metallic nanoparticles has attracted much attention owing to its unique characteristics, including the enhancement of signals in sensors and photothermal effects. In particular, hollow gold nanostructures are highly promising for practical applications, with significant advantages being found in their material properties and structures: 1) the interaction between the outer surface plasmon mode and inner cavity mode leads to a greater resonance, allowing it to absorb near-infrared light, which can readily penetrate tissue; 2) it has anti-corrosiveness and good biocompatibility, which makes it suitable for biomedical applications; 3) it shows a reduced net density and large surface area, allowing the possibility of nanocarriers for drug delivery. In this review, we present information on the classification, characteristics, and synthetic methods of hollow gold nanostructures; discuss the recent advances in hollow gold nanostructures in biomedical applications, including biosensing, bioimaging, photothermal therapy, and drug delivery; and report on the existing challenges and prospects for hollow gold nanostructures.

Keywords: localized surface plasmon resonance, hollow gold nanostructures, biomedical application, biosensors, bioimaging, photothermal therapy

INTRODUCTION

Owing to recent advances in the fabrication and analysis of metal nanoparticles, localized surface plasmon resonance (LSPR) has been extensively investigated and used in a broad range of fields. LSPR is the strong oscillation of the electron cloud of metal nanoparticles induced by incident light (Figure 1). This phenomenon increases the intensity of the electromagnetic field around the metal nanoparticles and is observed as a resonance absorption or scattering peak in the spectrum (Maier, 2007; Kreibig and Vollmer, 2013). The properties of LSPR can be affected by several factors, including the size and morphology of the nanoparticles, as well as the local dielectric environment (Jensen et al., 2000; Tran et al., 2020; Sekhon, 2021). By varying these factors, the LSPR peak can be tuned between visible and near-infrared light. The above-mentioned characteristics of LSPR allow us to apply it to various biomedical applications, such as biosensing (Lee et al., 2011), bioimaging (Feng et al., 2015), photothermal therapy (PTT) (Ding et al., 2014), and drug delivery (Bao et al., 2016).

Gold nanostructures, a candidate material for effective LSPR, have been widely researched for their biomedical applications because of their large surface-to-volume (S/V) ratio, biocompatibility, and ease of surface modification (Chithrani et al., 2006; Lim et al., 2011). Gold nanostructures can be classified into two groups according to their structural symmetry: 1) isotropic, such as gold...
nanospheres, and 2) anisotropic, such as gold nanorods, nanoprisms, and nanostars. These gold nanostructures have unique optical and physical properties.

Among the aforementioned gold nanostructures, hollow gold nanostructures, which have hollow cavities inside them, have been intensively investigated owing to their unique advantages over pre-existing solid gold nanostructures (e.g., larger S/V ratio, enhanced resonance with the interaction of outer–inner plasmons, and availability of internal cavities to encapsulate other substances, which have the potential to be exploited in various biomedical applications) (Skrabalak et al., 2007; Goodman et al., 2014). Herein, we summarize the classification, characteristics, and synthetic methods of hollow gold nanostructures by focusing on their advantages and potential. The research trends and prospects of hollow gold nanostructures in biomedical applications corresponding to biosensors, bioimaging, PTT, and drug delivery are presented.

CHARACTERISTICS AND SYNTHESIS OF HOLLOW GOLD NANOSTRUCTURES

Characteristics of Hollow Gold Nanostructures

Similar to conventional gold nanostructures, hollow gold nanostructures are promising materials for biomedical applications, owing to their chemical stability, biocompatibility, and ease of surface modification. However, the most apparent difference between solid and hollow gold nanostructures is the existence of an inner cavity. The inner cavity provides additional functionalities to hollow gold nanostructures, such as anticancer drug loading and interaction between the inner and outer surfaces; thus, these structures can be utilized as a new strategy for chemo–photothermal therapy or light-responsive drug release systems. Hollow gold nanostructures can be synthesized with various sizes and morphologies (including hollow gold nanospheres, gold nanocages/frames, gold nanorings, hollow gold nanostars, hollow gold nanorods, and hollow gold nanoprisms) depending on several factors, including the synthetic protocol, reaction temperature, and reaction atmosphere (Figure 2). Although there are differences in morphology, these hollow gold nanostructures have a thin gold shell in common, and the LSPR characteristics can be tuned by controlling the shell properties, which is not the case for solid gold nanostructures.

Lindley et al. (2018) reported an improved size control method for Co$_2$B nanoparticles and the role of environmental oxygen for galvanic exchange, which gives two-fold tunability of hollow gold nanospheres. They synthesized two types of hollow gold nanospheres with different diameters and shell thicknesses: 1) identical outer diameters with different aspect ratios (the ratio of outer diameter to shell thickness), providing LSPR peak tunability (Figures 3A,B), and 2) different outer diameters with the same aspect ratio, providing identical LSPR peaks that allow a range of hollow gold nanoparticles to have uniform energy distributions (Figures 3C,D). Specifically, this two-fold tunability is caused by the hybridization of plasmons, which is the interaction between the outer- and cavity-surface plasmons. Prodan et al. (2003) reported the highly geometry-dependent plasmon response of hollow gold nanospheres based on the hybridization of plasmons. The outer and inner plasmons interact with each other owing to the finite thickness of the hollow gold nanosphere shell; therefore, this interaction can be controlled by changing the thickness. The above-mentioned results show that hollow gold nanostructures provide an additional factor (thickness of the shell layer) to control the LSPR properties, and these hollow structures are more viable than conventional solid gold nanostructures for various applications that require a certain particle size or specific LSPR peaks.
Synthesis of Hollow Gold Nanostructures

Metal-Template-Based Synthesis (Galvanic Replacement)

Hollow gold nanostructures—even the same structures—have been synthesized by various methods; however, synthesis using galvanic replacement is significantly dominant (Xia et al., 2013). Galvanic replacement is driven by the difference in electrochemical potential between the solid metal (regarded as A), which has a lower reduction potential, and another metal ion (regarded as B⁺), which has a higher reduction potential. While the solid metal with the lower potential is oxidized and transformed into a metal ion (from A to A⁺), the metal ion with the higher potential is reduced and transformed into a solid metal (from B⁺ to B) at the contact surface. Through these processes, hollow nanostructures can be successfully synthesized with several characteristics: 1) tunable elemental compositions (Lu et al., 2007b), 2) easily controllable porous shells (by varying the amounts of precursors for galvanic replacement) (Sun and Xia, 2004), 3) closely similar shapes to sacrificial templates (Chen et al., 2006), 4) short reaction time (~30 min) (Cobley and Xia, 2010), and 5) high S/V ratio (Oh et al., 2013). For hollow gold nanospheres and gold nanocages/frames, the typical synthetic methods use cobalt nanoparticles and silver nanocubes as sacrificial templates, respectively (Figures 4A,B). Liang et al. (2005) reported uniformly synthesized hollow gold nanospheres using cobalt nanoparticles as sacrificial templates for galvanic replacement; this synthetic method, with minor modifications, has been widely used to fabricate hollow gold nanospheres. The cobalt nanoparticle sacrificial templates were first synthesized by reducing Co²⁺ with NaBH₄ under a nitrogen atmosphere to avoid oxidation. The hollow gold nanostructures were then fabricated as Au⁺ and reduced onto the cobalt nanoparticles based on galvanic replacement and finally stirred under ambient conditions to ensure complete oxidation of the residual cobalt nanoparticles. The shape of the final hollow gold nanostructure

![Figure 3](image-url)
depends on the shape of the sacrificial template employed, as well as the post-treatment processes. Sun and Xia (2004) and Lu et al. (2007a) reported gold nanocages and gold nanoframes using silver nanocubes as a sacrificial template. In this process, gold-silver nanoboxes are first fabricated as HAuCl₄ and reduced onto the silver nanocubes, then gold nanocages or gold nanoframes are synthesized depending on the following processes: 1) further galvanic replacement by HAuCl₄ (path [I] in Figure 4B, gold nanocages) or 2) selective removal of silver using wet etchants such as Fe(NO₃)₃ and NH₄OH (path [II] in Figure 4B, gold nanoframes).

The hollow structures were confirmed using transmission electron microscopy (TEM). In addition to nanospheres (Figure 5A; cobalt nanoparticle-templated) and nanocages (Figure 5B; silver nanocube-templated), more complex hollow gold nanostructures, such as nanorods (Figure 5C; tellurium nanorod-templated) and octahedral nanoframes (Figure 5D; silver nanoparticle-templated), were successfully synthesized with uniform size and morphology by galvanic replacement. Unlike hollow gold nanospheres and nanocages/frames, hollow gold nanorods are fabricated using a new metallic sacrificial template; tellurium nanorods (Cai et al., 2018). They reported the synthesis of hollow gold nanorods with controllable aspect ratios using a selenium-doped tellurium nanorod-templated method for the first time. In addition to the hollow gold nanostructures in Figure 5, other hollow gold nanostructures, including nanostars (Zhu et al., 2020; cooper nanoparticle-templated), nanorings (Prieto et al., 2014; cobalt nanoparticle-templated), and nanoprisms (Aherne et al., 2010; silver nanoprisms-templated) can also be synthesized by galvanic replacement. It is worth noting again that galvanic replacement is the major approach for the synthesis of hollow gold nanostructures.

Non-galvanic Replacement-Based Synthetic Methods

Although galvanic replacement is a major synthetic method for hollow gold nanostructures, there are some drawbacks, such as 1) fragmentation or aggregation due to excessive galvanic replacement processes (Lee et al., 2014) and 2) the generation of insoluble byproducts that can be deposited on the surface of nanoparticles (Zhang et al., 2012). To overcome these issues, several other approaches have been tested as alternatives to galvanic replacement.

Wang et al. (2013) reported the successful fabrication of various hollow gold nanostructures, such as nanospheres,
nanocapsules, and elongated nanocapsules, by employing a facile soft template approach (Figure 6A). Here, a cationic gemini surfactant, hexamethylene-1,6-bis(dodecyl dimethylammonium bromide) (C₁₂C₆C₁₂Br₂), played an important role as a soft template. C₁₂C₆C₁₂Br₂ is induced to self-assemble into various forms of vesicles, such as capsule-like or tube-like aggregates, depending on its concentration. Moreover, it provides structural stability during reduction because of its strong aggregation tendency. Wang H. et al. (2015) reported the synthesis of gold nanocages and hollow gold nanospheres from polystyrene (PS)@Au core–shell nanospheres using solvent thermal treatment in N,N-dimethylformamide (DMF) (Figure 6B). Specifically, PS@Au core–shell nanospheres were synthesized by attaching gold seeds to PS surfaces, which were further modified with branched polyethyleneimine to enhance the absorption of gold seeds onto the PS surfaces. Then, gold nanocages and hollow gold nanospheres were fabricated by removing the PS templates of the PS@Au core–shell nanospheres with thermal treatment in DMF, and the morphologies were affected by the core–shell structures or the temperature and time of thermal treatment.

**BIOMEDICAL APPLICATIONS OF HOLLOW GOLD NANOSTRUCTURES**

Recently, inert and relatively biocompatible gold nanostructures have been adopted for various biomedical applications owing to their attractive chemical and physical properties (Tiwari et al., 2011; Ren et al., 2015; Zangabad et al., 2017). Gold nanostructures have significant potential benefits in biomedical and industrial applications, although they can have harmful effects on biological systems such as bioaccumulation, bioclearance, metabolism, and elimination. To resolve these limitations, many studies have been conducted to mitigate and improve adverse influences through modifications including diameter, coating, shape,
Among these families of gold nanostructures, hollow gold nanostructures are one of the most promising platforms in biomedical applications owing to their unique characteristics (Ko et al., 2013; Han et al., 2018). They are characterized by compact sizes of approximately 20–500 nm, simple and precise LSPR adjustments, and large absorption cross-sectional area per unit volume. These characteristics allow hollow gold nanostructures to be mass-produced in high quality with the capacity to load and release drugs owing to their hollow interiors.

Hollow gold nanostructures are also viable in complex in vivo environments owing to their high mechanical flexibility.
and stability; they can be used as suitable platforms for a variety of purposes, such as labeling with various ligands, biomolecules, and functionalization because of their atomically flat surfaces (Satija et al., 2015; Ye et al., 2020).

Table 1 briefly shows recent studies in hollow gold nanostructures that can be used in various biomedical applications. Therefore, in this section, we summarize three types of biomedical applications using hollow gold nanostructures: biosensors (diagnosis), PTT, and bioimaging applications (theragnostics) (Figure 7).

### Table 1 | Summary of recent studies in hollow gold nanostructures for biomedical applications.

| Structure type  | Synthetic method | Sacrificial template | References                        |
|-----------------|------------------|----------------------|------------------------------------|
| **Biosensor**   |                  |                      |                                    |
| Sphere          | Galvanic replacement | Co nanoparticle     | Azadmehr and Zarei. (2020); Choi et al. (2020) |
| Cage            | Galvanic replacement | Ag nanoparticle     | Lee et al. (2019)                 |
| Sphere          | Galvanic replacement | Ag nanocube         | Chen et al. (2021)                |
| Cage            | Galvanic replacement | Cobalt nanoparticle | Lindley and Zhang (2019); Shen et al. (2020) |
| Rod             | Galvanic replacement | Ag nanoparticle     | He et al. (2021)                  |
| Star            | Galvanic replacement | Ag nanoparticle     | Fang et al. (2020)                |
| Rod             | Galvanic replacement | Te nanorod          | Zhang et al. (2019)               |
| **PTT**         |                  |                      |                                    |
| Sphere          | Galvanic replacement | Ag-SiO2 nanoparticle | Kim et al. (2020)                |
| Cage            | Galvanic replacement | Cu nanoparticle     | Zhu et al. (2020)                 |
| Biomedical imaging |                  |                      |                                    |
| Sphere          | Galvanic replacement | Co nanoparticle     | Cui et al. (2020)                 |
| Cage            | Non-galvanic replacement | Ag nanocube      | Depuch et al. (2020)             |
| Rod             | Galvanic replacement | TeSe nanorod       | Gao et al. (2020)                 |
| **Bioimaging**  |                  |                      |                                    |
| Sphere          | Galvanic replacement | Ag nanoparticle     | Wang et al. (2020)                |

### Biosensor: Diagnostics Application

Biosensors have been developed for various analyses across the fields of medicine, food testing, environmental sensing, and process control monitoring for research and industry. It is defined as a transducer that includes biological recognition components and consists of a three-factor system of bioreceptors, transducers, and signal processing devices (Conroy et al., 2009; Goode et al., 2015; Yoon et al., 2013). The distinct LSPR property is one of the most representative features of gold nanoparticles, and the peak position of LSPR is...
FIGURE 8 | miRNA-detection biosensor for acute myocardial infarction diagnosis. (A) Target-catalyzed hairpin assembly process and final structure for the surface-enhanced Raman spectroscopy detection platform. (B) Polyacrylamide gel electrophoresis results of the miR-133a-powered DNA molecular machine. (C) Representative SERS spectra corresponding to miR-133a with varying concentrations. Biosensor to detect brain damage-related proteins. (D) Illustration of SERS-based immunoassay for neuron-specific enolase (NSE) and S100 calcium-binding protein beta (S100-β) detection. (E) SERS spectra of the multiplex immunoassay after incubation with various concentrations of S100-β and NSE. Biosensor utilizing DNA to detect avian influenza virus. (F) Schematic image of the (Continued)
affected by the morphology, size, and surrounding medium of the nanoparticles; in particular, it is closely related to the refractive index (RI) of the material close to the nanoparticle surface. Because LSPR relies on RI around nanoparticles, it can be the basis for detecting interactions with molecules near nanoparticle surfaces (Ko et al., 2015; Zhang et al., 2018). The scattering spectrum of a single plasma resonance nanoparticle provides a single molecular event, and for nanoparticles, it can have a high RI sensitivity limited to nanometer-scale sensing volumes surrounding pointed tips. The LSPR-based biosensor uses a mechanism to observe noticeable changes in the peak wavelength of plasma resonance when a single giant molecule, such as a protein with a RI different from that of water, enters or leaves this sensing volume. Hollow gold nanostructures have higher RI sensitivities than solid gold nanostructures (Mayer et al., 2010; Ren et al., 2015; Zeng et al., 2020; Kim et al., 2020).

Detect of disease-related biomarkers plays an important role in the diagnosis and treatment. Among the various biosensors, surface-enhanced Raman spectroscopy (SERS)-based biosensor is one of the most powerful spectroscopic tools for biomedical field with high sensitivity and specific fingerprints and fast data collection speeds (Sutter et al., 2020; Cai et al., 2020b; Chung et al., 2015; Jeong et al., 2019). A hollow silver/gold nanosphere-based SERS probe was developed by Sun and Li (2018) for the diagnosis and treatment of acute myocardial infarction (AMI), a severe cardiovascular disease. This hollow nanostructure enables the ultra-high-sensitivity detection of AMI-related miRNA (miR-133a) using a target-catalyzed hairpin strategy. In this process, the target miRNA was used directly as a linker to capture SERS-based probes in sandwich mode, and a duplex linker with two sticky ends was selected in the target catalyst hairpin assembly strategy (Figures 8A–C). Polyacrylamide electrophoresis experiments were then performed to verify that hairpin DNA 1 and 2 (hp1 and hp2) were consumed to generate targets in SERS analysis and that miR-133a was used as a linker to hold probes on the substrate. Consequently, the presence of miR-133a became increasingly apparent as the new band moved slowly and increased in concentration, and the hp1, hp2 mixture disappeared from lanes to 6–8 (Figure 8B). In the SERS spectrum of miR-133a at various concentrations, the strength of the peak appeared to increase with increasing Raman dye (nitroblue tetrAzolium) concentrations (Figure 8C) (Sun and Li, 2018).

For protein detection, an immune sensor to diagnose brain damage by detecting neuron-specific enolase (NSE) and S100-b proteins in the blood was developed (Wang et al., 2018). The active group of hollow gold nanospheres and the redox molecules 4-mercaptobenzoic acid (4-MBA) and Nile blue A incorporate antibodies and provide signals to design a simple label-free threedimensional hierarchical plasmonic nanostructure (Figures 8D,E). In the presence of protein biomarkers, sandwich nanoparticles are captured on a substrate to develop a finite plasma field that amplifies Raman signals. Thus, as the S100-b and NSE protein concentrations increased, the peaks in the SERS spectrum became stronger (Figure 8E). In the case of hollow gold spike-like nanoparticles, a biosensor was also reported to detect avian influenza virus (AVI) with the introduction of a multifunctional DNA 3 way-junction (Figures 8F,G) (Lee et al., 2019). To detect AVI, each piece of the DNA 3 way-junction was adjusted to a hemagglutinin (HA) binding aptamer, 5′-fluorescein phosphoramidite (FAM) dye, or thiol group, respectively. In this system, the hollow gold spike-like gold nanoparticles were fixed to the ITO substrate to measure LSPR and immobilized onto the electrode via the thiol group. The fabricated biosensor detected HA proteins in phosphate-buffered saline (limit of detection (LOD): 1 pM) and diluted chicken serum (LOD: 1 pm) and confirmed that the LSPR peak increased as the concentration increased from 1 pM to 100 nM (Figure 8G).

Photothermal Therapy: Therapeutic Application

PTT is an effective cancer treatment method using nanoparticles that utilize a mechanism in which light is absorbed by nanoparticles and converted into heat within tumor tissues. When the local temperature in tumor tissue reaches 42–47°C, tumor cells—owing to their heat sensitivity being higher than that of normal cells—quickly undergo necrosis (Farokhnezhad and Esmaeilzadeh, 2019). This treatment is characterized by minimal invasiveness and excellent tissue penetration. Gold nanoparticles have received much attention because of their unique photothermal conversion properties and LSPR properties (Bi et al., 2019; Ling et al., 2021). The high affinity of tumor cells for the applied gold nanoparticles should have an efficient photothermal effect, so appropriate receptors or ligands should be modified on the particle surface to enable tumor-specific targeting (Grabowska-Jadach et al., 2019; Xu et al., 2021).

Gold nanostructures can be used to treat obesity. Their easy facile modification, superior stability, nontoxicity and biocompatibility make this possible. In addition, the properties of absorbing visible and near-infrared light to convert to thermal energy lead to effective photothermal treatment. Figures 9A,B achieves non-invasive photothermal ablation targeting abnormal adipose tissue through hyaluronic acid and peptides, and hollow gold nanosphere (Lee et al., 2017). This is transmitted through the subcutaneous, efficiently targeted at adipocytes, and enables highly effective photothermal ablation with NIR laser. The successful results were reported in Figure 9B through experiments in C57BL/6 obesity mice. After topical treatment of hollow gold nanosphere conjugated with hyaluronic acid and
FIGURE 9 | Transdermal hyaluronic acid-hollow gold nanosphere-ATP conjugate for obesity treatment. (A) Schematic illustration of the targeted photothermal lipolysis of adipocytes after noninvasive transdermal delivery. (B) Photoacoustic images of subcutaneous adipose tissues before and after NIR lasers. Hollow gold nanoflowers with strong photothermal effects in near-infrared (NIR) areas. (C) Schematic representation showing the photothermal effect of the gold nanoflowers (AuNFs) on endocytosis under NIR irradiation. (D) Photothermal effect on a nude mouse under NIR irradiation caused by the addition of gold nanoflowers. (E) Relationship between HeLa cell viabilities and the concentration of gold nanoflowers with laser irradiation (λ = 808 nm, 9 W/cm², 5 min). Gold nanocages (AuNC) loaded (Continued)
ATP in the abdominal region of mice, NIR lasers were investigated for photothermal lipolysis for 10 min. As a result, the significant reduction of photoacoustic signals in adipose tissue in the dotted area confirmed effective photothermal lipolysis, and no side effects were shown.

One of the advantages of hollow gold nanostructure is that it is easy to control LSPR, which can be adjusted in size of nanostructure from the visible light field to the near-infrared field for cancer photothermal therapy. Figures 9C–E shows a study that produced a hollow gold nanoflower structure to indicate high biological stability in visible light and very strong photothermal cytotoxicity to tumor cells in near-infrared environments (Han et al., 2014). Experiments were conducted with hollow gold nanoflowers using surfactant bis-(amidethylocarbamoyloethyl) octadecylamine (C18N3) as templates with multi-amine head groups on a tumor-bearing mouse model. Therefore, tumor size increased in the control group. However, the tumor almost disappeared from the lesion after 20 days in the gold nanoflower-treated mice (Figure 9B).

Figure 9E shows a substantial decrease in HeLa cell viability affected by 808 nm NIR laser, size, and concentration of gold nanoflowers.

Hollow gold nanostructures can be used to treat bacterial infections and cancers. Studies have demonstrated the synergistic treatment of bacterial infections by loading antibiotic daptomycin (Dap) into polydopamine (PDA)-coated gold nanocages (Figures 9F, G) (Meeker et al., 2016). To induce target binding to bacterial cell surface proteins (Spa) via anti-Spa, PDA was coated onto the surface and the nanostructure was activated by near-infrared radiation to convert photon energy into thermal energy, increasing the photothermal effect and degenerating the PDA coating to release the antibiotics (Figure 9F). Figure 9G shows the results of antibiotic treatment on 0.5 cm segments of catheter biofilm. Exposure to 5 µg/ml Dap in groups 1 and 2 had a slight effect on the number of viable bacteria per catheter compared to that in the control group, where nothing was processed. Exposure to untargeted gold nanocage@PDA after laser irradiation showed an insignificant decrease in the number of viable bacteria per catheter from 0 to 24 h (Group 3). With aSpa, the photothermal effect was increased immediately after irradiation on targeted gold nanocage@PDA, but it can be seen to increase from 24 h because of its failure to completely exterminate viable bacteria in the biofilm (Group 4). In group 5, a clear photothermal effect was observed shortly after laser irradiation of bacteria exposed to gold nanocage@Dap500/PDA—aSpa, and no viable bacteria were detected at 24 h. In addition, bacteria were not detected at either 0 or 24 h when the biofilm was laser-irradiated by exposure to gold nanocage@Dap500/PDA—aSpa. These results confirmed the synergistic effects of photothermal killing and targeted antibiotic release.

Hollow gold nanostructures have high photothermal conversion effects and excellent biocompatibility, can adjust the LSPR band in near-infrared (NIR) regions, and are suitable for drug delivery. The irradiation of gold nanoparticles with NIR lasers can accelerate the release of cargo by generated hyperthermia to the target location and ablate the tumor cells with favorable PTT (Gan et al., 2019; Sun et al., 2021).

By combining PTT with encapsulating drugs in the internal space of hollow gold nanostructures where LSPR control is easy, improved therapeutic results can be achieved compared to the existing method. But the innate structures of the hollow gold nanostructure can leak drugs early, which makes decreases targeting efficiency. To prevent this, utilizing biomimetic cell membrane-based drug delivery systems can significantly increase the stability of nanoparticles and prevent drug leakage. Research has been conducted using cancer cell membranes coated with gold nanocages to suppress breast cancer metastasis (Figures 10A–C) (Sun et al., 2017). In chemotherapy, nanostructures coated with biomimetic cell membranes can significantly increase stability with fewer drug leaks under physiological conditions. Light heat at 43°C can also increase the permeability and fluidity of the cell membrane, thereby improving the intracellular accumulation of drugs. Cancer cell membrane-coated gold nanocages were fabricated by loading the anticancer drug Doxorubicin (DOX) into the inner core of gold nanocages and then coating with the cancer cell membrane derived from 4T1 breast cancer cells. A sulforhodamine B assay to determine the antiproliferative effect of gold nanocages showed that the strongest antiproliferative effect was on tumor cells cultured with cancer cell membrane-coated gold nanocages, resulting from the accelerated release of DOX from gold nanocages by laser-induced high heat and induced phototoxicity (Figure 10B). The in vivo inhibitory effect of cancer cell membrane-coated gold nanocages on tumors and pulmonary metastasis is shown in Figure 10C. Evaluation of the 4T1 tumor transition model showed rapid tumor growth in the saline treatment group and excellent antitumor effects in the NIR investigation group.

A minimally invasive transdermal drug delivery system is available to minimize the side effects and pains (Li et al., 2017; Pagneux et al., 2020). To achieve this goal, hyaluronic acid dissolving microneedle (MN) arrays were developed utilizing gold nanocages loaded with DOX (Figures 10D–F) (Dong et al., 2018). The fabricated MNs effectively penetrate the skin, dissolve, release drugs within the tumor, and exhibit photothermal and synergistic effects by near-infrared laser.
FIGURE 10 | Gold nanocages that suppress high breast cancer metastasis by coating cancer cell membranes. (A) Schematic illustration of the 4T1 cancer cell membrane-coated gold nanocage for the hyperthermia-triggered release of doxorubicin (DOX) and homotypic targeted therapy of breast tumor growth and metastasis. (B) Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) staining (200×) of the tumor tissues, the collected lungs, and H&E staining (Scale bar = 100 µm) of the lung tissue. White arrows indicate the metastatic models. (C) In vivo antitumor and antimetastasis effects of the nanoparticles in the 4T1 tumor-bearing nude mice of tumor growth profiles. Example of applying gold nanocages to microneedles for the local treatment of superficial skin tumors. (D) (Continued)
irradiation. The confocal laser scanning microscope (CLSM) provides a visual view of the release of gold nanocages perpendicular to the surface of the mouse skin (Figure 10E). In addition, red fluorescent arrays of gold nanocage spots were clearly detected in the skin. Fluorescent signals were strongly maintained between 150 and 230 μm and then decreased slowly, and the red spot in the CLSM and the black spot in the light field image almost overlapped in the merged image. This indicates that the released gold nanocages are directly inside the insertion path and only a small fraction spread into the surrounding tissue. Figure 10F shows the high tumor growth inhibition effect of gold nanocages with the simultaneous application of NIR and DOX within tumors.

Biomedical Imaging: Theranostic Application

In general, gold nanoparticles are potentially applicable to a wide variety of fields in optical imaging, cell imaging, ultrasound imaging, and positron emission tomography. Biomedical imaging is a non-invasive functional imaging technology that detects and monitors various human physiological and
pathological conditions. Its use allows the early detection of tumors and other diseases, and guides precision treatment. Gold nanoparticles are well known for their ability to increase biological inactivity and spatial and temporal resolution for imaging (Singh et al., 2018; García-Álvarez et al., 2020; Si et al., 2021; Qambrani et al., 2021).

Hollow gold nanostructures can be used in various biomedical imaging fields, such as TEM, computed tomography, SERS imaging, and photoacoustic tomography (PAT). (Nagy-Simon et al., 2017) reported a new class of contrast medium containing NIR-reactive hollow gold nanospheres for the detection and multimodal imaging of CD19 (+) cancer lymphocytes (Figure 11A). The medium binds to an anti-CD19 monoclonal antibody based on hollow gold nanospheres and is denoted by the SERS active molecule Nile blue to complete the hollow gold nanosphere–Nile blue–PEG–anti-CD19 complex. TEM was used to investigate the specificity of this contrast medium, and dark-field, SERS, and two-photon excitation fluorescence lifetime imaging microscopy proved its interaction with TEM and the preferential internalization of the complex. Therefore, improvements in the SERS background occurred over the entire measurement interval and could only be observed in local areas corresponding to the accumulated nanoparticles. In conclusion, (Nagy-Simon et al., 2017) demonstrated that antibody complexes provide labeling molecules and SERS background signals for internalization and intracellular positioning, and are promising materials for non-invasive microscopic imaging.

PAT, also referred to as optoacoustic tomography or photoacoustic imaging, is a powerful nonionizing optical imaging modality that integrates the advantages of both optical radiation and ultrasonic detection (Yao and Wang, 2011; von Knorring and Mogensen, 2020). This imaging technology can be monitored with an optical contrast agent, such as gold, which facilitates high sensitivity and specificity, separating the signal contributions of several optical absorbers by spectroscopy, enabling simultaneous molecular and functional imaging. Recently, hollow gold nanospheres have emerged as novel molecular contrast agents for PAT; they can be adjusted to the near-infrared range, show strong resonance absorption and absorption peaks at ~800 nm, and allow surface coating with thiolated polyethylene glycol (PEG), which leads to significantly higher PA efficiency (Lu et al., 2010). The PEGylated hollow gold nanospheres clearly expressed a small cerebral vascular structure with a diameter of ~100 nm in PAT images obtained within 2 h of intravenous administration of nude mice (Figure 11B); however, preliminary results have not shown acute toxicity to the liver, spleen, or kidneys of mice, and consequently show promise as contrast agents for PAT with relatively high spatial resolution and improved sensitivity.

These bioimaging applications can be combined with therapeutic applications, such as drug delivery and PTT, to simultaneously perform treatment and diagnosis to enable precision medicine that can be tailored to each patient (Moorkoth et al., 2021; Yang et al., 2021). Nanoscale particles can be targeted to desired positions through surface functionality (Wijetunga et al., 2020). In particular, the utilization of hollow gold nanostructures can enhance the efficiency of theranostics owing to their inherent characteristics. For example, nontoxic hollow gold nanorods can be used as chemo–photothermal therapy substances at the same time and as contrast agents for multimodal imaging for cancer theranostics (Figure 11C) (Cai et al., 2018). The platform is a new type of nano-agent with LSPR peaks in the NIR-II window (1,000–1,350 nm), which is more suitable for biological applications owing to its deeper tissue penetration capabilities and higher maximum permissible exposure than that of the NIR-I window (700–950 nm). Figure 11C demonstrates the ability of the computed tomography imaging contrast agent by injecting hollow gold nanorods into a tumor-bearing mouse to confirm the brightness in the tumor region. After loading DOX, the laser was examined to confirm the chemo–photothermal effect. Although the data are not indicated in this paper, when phototherapy and chemotherapy were applied at the same time, the tumor disappeared completely after approximately 10 days, confirming the synergistic effect. Thus, it can be seen that hollow gold nanostructures show improved ability when applied with PTT as well as bioimaging.

CONCLUSIONS AND FUTURE PERSPECTIVES

Hollow gold nanostructures have drawn attention for their biomedical applications because of their unique physical and optical properties, such as facile LSPR tunability, drug loading capacity, and enhanced photothermal activity, compared to those of conventional solid gold nanoparticles. In this review, we summarized the recent research trends in hollow gold nanostructures, including synthetic methods, physical/optical characteristics, and recent advances in biomedical applications. Various forms of hollow gold nanostructures can be synthesized by either hard- or soft-templating methods, but the majority of synthesis is based on galvanic replacement. While the LSPR peaks of solid gold nanoparticles depend solely on the overall size of the particles, those of hollow gold nanostructures are greatly influenced not only by the size, but also by the thickness of the shell. Therefore, the LSPR peaks can be easily adjusted while maintaining the overall size of the particles, which provides additional tunability of the SERS signal or photothermal activity. The voids in the interior also provide additional functionality to hollow gold nanostructures by being utilized as nanoscale storage for impregnating functional materials (e.g., anticancer drugs), which is not possible in solid gold nanoparticles. Based on these characteristics, various hollow gold nanostructures have been widely used in several biomedical applications, including biosensors, photothermal therapy, and biomedical imaging. It should be noted that there have been many studies aimed at the synergistic effects of simultaneously utilizing the various functionalities of hollow gold nanostructures, such as combined chemo–photothermal therapy, light-responsive drug release, and plasmonic nanostructure-based bioimaging.
Despite this progress, there are still many issues: 1) off- and on-target accumulation of hollow gold nanostructures and 2) preventing the aggregation and disassembly of hollow gold nanostructures. Large-sized hollow gold nanostructures (larger than 200 nm) can lead undesirable accumulation in the reticuloendothelial system (e.g., liver, spleen, and lungs); on the other hand, very small-sized one cannot provide sufficient on-target accumulation of them due to the short circulation time. Even if the size of these structures is suitable for selective accumulation, the aggregation and disassembly of hollow gold nanostructures in human body can decrease the LSPR intensity. Therefore, researchers should consider 1) appropriate size of nanostructures for sufficient on-target accumulation and prevention of off-target side effect of them; 2) antibody conjugation which enhance the capability of target adhesion; and 3) the stability of hollow gold nanostructures in human body to provide good LSPR properties. We strongly believe that the above-mentioned problems will be overcome by further research using advanced synthetic methods or surface modifications in the future; then, hollow gold nanostructures will become increasingly important in biomedical applications because it is clear that these unique structures have many advantages compared to conventional nanoparticles. We hope that this review will help readers to comprehend the recent advances in hollow gold nanostructures and will provide great inspiration for researchers.

AUTHOR CONTRIBUTIONS

Conceptualization, J-HK and KK; investigation, J-MP, HC, J-HK, and KK.; writing—original draft preparation, J-MP, J-HK, DC, J-HK, and KK; writing—review and editing, J-HK and KK; visualization, J-MP and HC and KK; supervision, J-HK and KK; funding acquisition, J-HK and KK. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

Aberne, D., Gara, M., Kelly, J. M., and Gun’ko, Y. K. (2010). From Ag Nanoprisms to Triangular AuAg Nanoboxes. Adv. Funct. Mater. 20, 1329–1338. doi:10.1002/adfm.200902030
Azadmehr, F., and Zarei, K. (2020). Ultrasensitive Determination of Cefixime Using Pencil Graphite Electrode Modified by Hollow Gold Nanoparticles/reduced Graphene Oxide. Arab J. Chem. 13, 1890–1900. doi:10.1016/j.ajche.2018.02.004
Bao, T., Yin, W., Zheng, X., Zhang, X., Yu, J., Dong, X., et al. (2016). One-pot Synthesis of PECoated Plasmonic MoO3-X Hollow Nanospheres for Photocatalytic Imaging Guided Chemo-Photothermal Combinational Therapy of Cancer. Biomaterials. 76, 11–24. doi:10.1016/j.biomaterials.2015.10.048
Bi, J., Cai, H., Wang, B., Kong, C., and Yang, S. (2019). Localized Surface Plasmon Enhanced Electrocataytic Methanol Oxidation of AgPt Bimetallic Nanoparticles with an Ultra-thin Shell. Chem. Commun. 55, 3943–3946. doi:10.1039/C9CC03311B
Cai, K., Zhang, W., Foda, M. F., Li, X., Zhang, J., Zhong, Y., et al. (2020a). Miniature Hollow Gold Nanorods with Enhanced Effect for In Vivo Photocatlocic Imaging in the NIR-II Window. Small 16(37), 2002748. doi:10.1002/smll.202002748
Cai, K., Zhang, W., Zhang, J., Li, H., Han, H., and Zhai, T. (2018). Design of Gold Hollow Nanorods with Controllable Aspect Ratio for Multimodal Imaging and Combined Chemo-Photothermal Therapy in the Second Near-Infrared Window. ACS Appl. Mater. Inter. 10, 36703–36710. doi:10.1021/acsami.8b12758
Cai, Z., Hu, Y., Sun, Y., Gu, Q., Wu, P., Cai, C., et al. (2020b). Plasmonic SERS Biosensor Based on Multibranched Gold Nanoparticles Embedded in Polydimethylsiloxane for Quantification of Hematin in Human Erythrocytes. Anal. Chem. 93, 1025–1032. doi:10.1021/acs.analchem.9c03921
Chen, J., McLellan, J. M., Siekkinen, A., Xiong, Y., Li, Z.-Y., and Xia, Y. (2006). Facile Synthesis of Gold–Silver Nanocages with Controllable Pores on the Surface. J. Am. Chem. Soc. 128, 14776–14777. doi:10.1021/ja066023g
Chen, M., Wu, D., Tu, S., Yang, C., Chen, D., and Xu, Y. (2021). A Novel Biosensor for the Ultrasensitive Detection of the IncRNA Biomarker MALAT1 in Non-small Cell Lung Cancer. Sci. Rep. 11, 1–11. doi:10.1038/s41598-021-83244-7
Chithrani, B. D., Ghazani, A. A., and Chan, W. C. W. (2006). Determining the Size and Shape Dependence of Gold Nanoparticle Uptake into Mammalian Cells. Nano Lett. 6, 662–668. doi:10.1021/nl052396o

Choi, N., Dang, H., Das, A., Sim, M. S., Chung, I. Y., and Choo, J. (2020). SERS Biosensors for Ultrasensitive Detection of Multiple Biomarkers Expressed in Cancer Cells. Biosens. Bioelectron. 164, 112326. doi:10.1016/j.bios.2020.112326
Chung, E., Jeon, J., Yu, J., Lee, C., and Choo, J. (2015). Surface-Enhanced Raman Scattering Aaptosensor for Ultrasensitive Trace Analysis of Bisphenol A. Biosens. Bioelectron. 64, 560–565. doi:10.1016/j.bios.2014.09.087
Cobley, C. M., and Xia, Y. (2010). Engineering the Properties of Metal Nanostructures via Galvanic Replacement Reactions. Mater. Sci. Eng. R: Rep. 70, 44–62. doi:10.1016/j.mser.2010.06.002
Conroy, P. J., Hearty, S., Leonard, P., and O’Kennedy, R. J. (2009). Antibody Production, Design and Use for Biosensor-Based Applications. Semin. Cell Developmental Biol. 20, 10–26. doi:10.1016/j.semcdb.2009.01.010
Cui, G., He, P., Yu, L., Wen, C., Xie, Y., and Yao, G. (2020). Oxygen Self-Enriched Nanoplatform Combined with US Imaging and Chemo/photothermal Therapy for Breast Cancer. Nanomedicine: Nanotechnology, Biol. Med. 29, 102238. doi:10.1016/j.nano.2020.102238
Depciuch, J., Stec, M., Maximenko, A., Baran, J., and Parlinska-Wojtan, M. (2020). Temperature-controlled Synthesis of Hollow, Porous Gold Nanoparticles with Wide Range Light Absorption. J. Mater. Sci. 55 (12), 5257–5267. doi:10.1007/s10853-020-4345-8
Ding, X., Liow, C. H., Zhang, M., Huang, R., Li, C., Shen, H., et al. (2014). Surface Plasmon Resonance Enhanced Light Absorption and Photothermal Therapy in the Second Near-Infrared Window. J. Am. Chem. Soc. 136, 15684–15693. doi:10.1021/ja508641z
Dong, L., Li, Y., Li, Z., Xu, N., Liu, P., Du, H., et al. (2018). Au Nanocage-Strengthened Dissolving Microneedles for Chemo-Photothermal Combined Therapy of Superficial Skin Tumors. ACS Appl. Mater. Inter. 10, 9247–9256. doi:10.1021/acsami.7b18293
Fang, X., Lui, K.-H., Li, S., Lo, W.-S., Li, X., Gu, Y., et al. (2020). Multifunctional Nanotheranostic Gold Nanocage/Selenium Core-Shell for PAI-Guided Chemo-Photothermal Synergistic Therapy In Vivo. Ijn Vol. 15, 10271–10284. doi:10.1016/j.nano.2020.10271
Farokhzad, M., and Esmaelzadeh, M. (2019). Graphene Coated Gold Nanoparticles: an Emerging Class of Nanoagents for Photothermal Therapy Applications. Phys. Chem. Chem. Phys. 21, 18352–18362. doi:10.1039/C9CP03126f
Feng, A. L., You, M. L., Tian, L., Singamaneni, S., Liu, M., Duan, Z., et al. (2015). Distance-dependent Plasmon-Enhanced Fluorescence of Upconversion Nanoparticles Using Polyelectrolyte Multilayers as Tunable Spacers. J. Phys. Chem. B 119, 15693–15700. doi:10.1021/jp5106002
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Influenza Virus Detection. *Colloids Surf. B: Biointerfaces* 182, 110341. doi:10.1016/j.colsurfb.2019.06.070

Li, C., Ye, R., Bouchaert, J., Zurutuza, A., Drider, D., Dumyts, T., et al. (2017). Flexible Nanoholey Plates for Antibiotic-free Treatments of Skin Infections. *ACS Appl. Mater. Inter.* 9, 36663–36674. doi:10.1021/acsami.7b12949

Li, H.-P., Wan, L.-J., Bai, C.-L., and Jiang, L. (2005). Gold Hollow Nanoshapes: Tunable Surface Plasmon Resonance Controlled by interior-cavity Sizes. *J. Phys. Chem. B* 109, 7795–7800. doi:10.1021/jp054006f

Lim, D.-K., Jeon, K.-S., Hwang, J.-H., Kim, H., Kwon, S., Suh, Y. D., et al. (2011). Highly Uniform and Reproducible Surface-Enhanced Raman Scattering from DNA-Tailorable Nanoparticles with 1-nm interior gap. Nat. Nanotech. 6, 452–460. doi:10.1038/nnano.2011.79

Linley, S. A., Ho, J. K., Rojas-Andrade, M. D., Fung, V., Leshy, C. J., Chen, S., et al. (2018). Highly Tunable Hollow Gold Nanoshapes: Gaining Size Control and Uniform Galvanic Exchange of Sacrificial Cobalt Boride Scaffolds. *ACS Appl. Mater. Inter.* 10, 12992–13001. doi:10.1021/acsami.8b00726

Linley, S. A., and Zhang, J. Z. (2019). Bumpy Hollow Gold Nanospheres for Theranostic Applications: Effect of Surface Morphology on Photothermal Conversion Efficiency. *ACS Appl. Nano Mater.* 2, 1072–1081. doi:10.1021/acsanm.8b02331

Ling, C., Wang, X., and Shen, Y. (2021). Advances in Hollow Inorganic Nanomedicines for Photothermal-Based Therapies. *Jn Vol.* 16, 493–513. doi:10.1021/acs.jn.1c00119

Lopez-Chaves, C., Soto-Alvaredo, J., Montes-Bayon, M., Bettmer, J., Llopis, J., and Sanchez-Gonzalez, C. (2018). Gold Nanoparticles: Distribution, Bioaccumulation and Toxicity. In Vitro and In Vivo Studies. *Nanomedicine* 14 (1), 1–12. doi:10.2217/nnm.17.8011.0811

Lu, W., Huang, Q., Ku, G., Wen, X., Zhou, M., Guzatov, D., et al. (2010). Photocoustic Imaging of Living Mouse Brain Vasculature Using Hollow Gold Nanoshells. *Biomater. Biosci.* 31, 2617–2626. doi:10.1021/bm901359s

Luo, X., Xu, A., Mcellan, J., Li, Z.-Y., Marquez, M., and Xia, Y. (2007a). Fabrication of Cubic Nanocages and Nanoframes by Dealloying Au/Ag Alloy Nanobundles with an Aqueous Etchant Based on Fe(NO3)3 or NH4OH. *Nano Lett.* 7, 1764–1769. doi:10.1021/nl0708381

Luo, X., Chen, J., Skrabalak, S. E., and Xia, Y. (2007b). Galvanic Replacement Reaction: A Simple and Powerful Route to Hollow and Porous Metal Nanostuctures. *Proc. Inst. Mech. Eng. N: J. Nanomanufacturing Systems* 221, 1–16. doi:10.1243/17404399JNN111

Maier, S. A. (2007). *Plasmonics: Fundamentals and Applications.* New York: Springer.

Mayer, K. M., Hao, F., Lee, S., Nordlander, P., and Hafner, J. H. (2010). A Single Molecule Immunoassay by Localized Surface Plasmon Resonance. *Nanotechnology* 21, 255503. doi:10.1088/0957-4484/21/25/255503

Meeker, D. G., Jenkins, S. V., Miller, E. K., Beekman, K. E., Loughran, A. J., Powless, A., et al. (2016). Synergistic Photothermal and Antibiotic Killing of Biofilm-Associated *Staphylococcus aureus* Using Targeted Antibiotic-Loaded Gold Nanocoatings. *ACS Infect. Dis.* 2, 241–250. doi:10.1021/acsiinfecdis.5b00117

Moorkoth, D., Nampoothiri, K. M., Nagarajan, S., Ravindran Girija, A., Balasubramaniam, S., and Kumar, D. S. (2021). Star-shaped Polylactide Dipyridamole Conjugate to 5-fluorouracil and 4-epiperidinopiperidine Nanocarriers for Bioimaging and Dual Drug Delivery in Cancer Cells. *ACS Appl. Polym. Mater.* 3, 737–756. doi:10.1021/acsapm.0c01043

Nag-Simon, T., Tatar, A.-S., Cruciani, A.-M., Vulpoi, A., Jurj, M.-A., Florea, A., et al. (2017). Antibody Conjugated, Raman Tagged Hollow Gold Nanospheres: Multicolor Tumor Imaging and Drug Delivery in Breast Cancer Cells. *ACS Appl. Mater. Inter.* 9, 2115–21168. doi:10.1021/acsami.7b05145

Oh, M. H., Yu, T., Yu, S.-H., Lim, B., Ko, K.-T., Willinger, M.-G., et al. (2013). Galvanic Replacement Reactions in Metal Oxide Nanocrystals. *Science* 340, 964–968. doi:10.1126/science.1234751

Paguenx, Q., Ye, R., Chengnan, L., Barras, A., Hennuyer, N., Staels, B., et al. (2020). Electrothermally Driven Patches Delivering the Transepidermal Delivery of Insulin. *Nanosc Horiz.* 5, 663–670. doi:10.1039/c9nr00576e

Pietro, M., Arenal, R., Henrard, L., Gomez, L., Sebastian, V., and Arrueto, M. (2014). Morphological Tunability of the Plasmonic Response: from Hollow Gold Nanospheres to Gold Nanorings. *J. Phys. Chem. C* 118, 28804–28811. doi:10.1021/jp5096129
