Application of gold nanoparticles in photoacoustic imaging

Wen He¹, Xiaoxia Wang¹, Xing Gao¹*, Zaijun Lu², and Jibing Song³*

¹School of Chemistry and Bioengineering, Qilu Institute of Technology, Jinan, Shandong, 250200, China
²School of Chemistry and Chemical Engineering, Shandong University, Jinan, Shandong, 250100, China
³College of Chemistry, Fuzhou University, Fujian, Fuzhou, 350108, China
*Corresponding author’s e-mail: gaoxing-620@163.com and jibinsong@fzu.edu.cn

Abstract. Photoacoustic (PA) imaging is a noninvasive imaging mode that has rapidly developed in recent years for medical diagnosis and treatment by using contrast agents. Among them, gold nanomaterials (GNPs) offer a suitable platform for PA imaging, owing to their advantageous physical/chemical/biological properties and high-intensity absorption in the near-infrared (NIR) region. In this review, we firstly concentrate on the recent development of GNPs and their assemblies for PA imaging. Then, the future development in the research of GNPs as PA imaging contrast agents are discussed.

1. Introduction
PA imaging is a noninvasive medical imaging mode which combines the advantages of the excellent contrast of traditional optical imaging with high penetrability (up to 5-6 cm) of ultrasound imaging and has rapidly developed in recent years for biomedical diagnosis and treatment. The imaging system exploits PA effect that was primary reported in 1880 by Alexander Graham Bell [1]. Concisely, as the contrast agent absorbs the pulsed laser, the thermoelastic expansion of biological tissues will generate broadband sound waves at megahertz frequencies. These broadband ultrasonic waves can be found by ultrasound transducers on the tissue surface, and changed to electric signals which processed appropriately to obtain PA image [2]. Since there were no strong light sources and sensitive detectors at that time, until the late 1990s, PA imaging developed rapidly and was widely used in biomedical fields.

Contrast agents for PA imaging can be classified into endogenous and exogenous contrast agents depending on the source. Endogenous contrast agents, such as melanin and hemoglobin, offer functional and structural message for PA imaging. However, exogenous contrast agents, e.g. small molecule dyes, fluorophores, nanomaterials, which can be imaged at the cellular and molecular levels, are able to greatly enhance these imaging modalities. For the moment, owing to the specific size and shape dependent physiochemical properties, remarkable bio/chemical inertness, and the optical characteristics originated from the giant electromagnetic field near the surface of GNPs, GNPs have become one of the most prospective exogenous contrast agents for PA imaging[3-4].

Here, the recent development of GNPs and their assemblies in PA imaging are outlined. Then, the perspectives of GNPs for PA imaging, especially about their clinical translation, are discussed.

2. Classification of plasmonic gold nanoparticles (GNPs) and their assemblies
GNPs based on their Localized Surface Plasmon Resonance (LSPR) effect adjusted by different sizes, shapes, and couplings of the nanoparticles [5], have been actively used as contrast agents for PA imaging[6]. Besides the original gold nanospheres, in recent years, researchers have synthesized many complicated nanostructures[7] including gold nanorods[8-9], gold nanocages[10], gold nanostars[11], gold nanoshells[12], and so on (Figure 1).

![Figure 1. Scanning electron microscope and transmission electron microscope micrographs of gold nanospheres (a), gold nanorods (b), gold nanoprisms (c), gold nanocages (d), gold nanostars (e), gold nanoplates (f), gold nanodisks (g) and gold nanoshells (h) [4].](image)

By collecting the literature, it is found that there is not much research on original gold nanospheres (GNSPs) for PA imaging. Because the optical absorption spectrum of GNSPs, typically at approximately 520 nm, is not fall into the biological window (650-1100 nm), in which the least blood and tissue attenuation occurs[2]. However, by modification with polymers or magnetic materials, GNSPs have red-shifted absorption spectra and thus could offer stronger PA signals in vivo. Lu[13] synthesized poly(ethylene glycol)-coated GNSPs (40-50 nm). They displayed intensive resonance absorption adjusted to the NIR region, with a maximum absorption peak at 800 nm, and its PA efficiency is remarkably higher than that of blood. Bai[14] developed a contrast agent based on GNSPs and Fe₃O₄. Utilizing the outstanding magnetic properties of Fe₃O₄ and strong NIR absorption performance of GNSPs, such nanocomposites had been used for targeted PA imaging of cancer cells, with a red-shift to 830 nm.

Gold nanorods (GNRs) equipped with tunable NIR absorption behavior by increasing in particle aspect (length to width) ratio that has been proved by Jokerst[15-16], so they are the most potential PA imaging contrast agents. However, without changing the aspect ratio, Chen[17] reported the synthesis of miniaturized GNRs absorbing at 1,064 nm, a wavelength within the NIR-II window, which ensures low background noise from endogenous absorbers in tissue, that are 5-11 times smaller than regular-sized GNRs with a similar aspect ratio. Miniaturized GNRs generated 3.5 times stronger PA signals under nanosecond pulsed laser illumination and displayed obviously improvement in overall tumour PA signals by 4.5-fold compared to the large counterparts (Figure 2) [17]. These findings provide another strategy for the design of future PA contrast agents and have the potential to stimulate more PA molecular imaging applications in the NIR-II window.
GNRs synthesized by the laboratory method usually have a film coated with a surfactant on the surface, which inhibits the activity of biological cell because of active surface electrons. Moon[18] synthesized the reduced graphene oxide coated GNRs (r-GO-GNRs). Theoretical research shows that compared with naked GNR or GO-GNR, the enhanced electromagnetic field around r-GO-GNR is 4 times stronger. In addition, due to the extremely high PA amplitude in the 4-11 MHz operating frequency of ultrasonic transducers, r-GO-GNRs are expected to be promising deep tissue imaging probes. Furthermore, Wu[19] used traditional Chinese medicine acupuncture as an auxiliary method, and developed an effective method to use a combination of acupuncture and (polyethylene glycol)-coated GNRs (PEG-GNRs) as a composite contrast agent to enhance the contrast of PA imaging. Yan[20]designed a comprehensive platform, that combines extremely sensitive nucleic acid probes with polyethylenimine-modified GNRs, named as GNRs-PEI/FIRE. GNRs-PEI/FIRE, which has potential applications in the diagnosis and treatment of tumors, displayed higher target miRNA in vitro/in vivo detection sensitivity and enhanced PA imaging signals compared to controls.

For application of PA imaging to diagnosis, GNRs have been suggested as molecular probes because they show strong PA signals. Umehara[9]synthesized GNRs (G-NIs) with NI (2-nitroimidazole derivative) units on the surface, and characterized its characteristics, which can be used as an indicator of hypoxia in PA imaging. GNRs showed a powerful PA signal (Figure 3)[9]. Due to the reducing properties of intracellular reductase to NI units, the binding of NI units and nanoparticles causes them to accumulate in hypoxic cells and tissues. Therefore, G-NIs are allowed to accumulate in hypoxic cells to display their PA signals in hypoxic tumor cells and tissues. G-NIs is a promising PA nanoprobe that can be used to track tumor hypoxia.
GNPs tied to polymer brushes were widely used as building blocks for building functional assemblies with various structures (Figure 4a) [21] as a result of the unique collective characteristics of GNPs assemblies that have multiple advantages comparing to the single GNPs. Due to the strong plasmon coupling effect between adjacent GNPs of the module, the LSPR spectrum often appears red-shifted, and the PA signal is greatly enhanced.

Among GNPs assemblies, gold nanovesicles (GNVs) have been widely used in biomedical applications [22]. As shown in Figure 4b, they can be formed by self-assembly. They can be formed by self-assembly of amphiphilic polymer grafted with GNPs [23]. As mentioned earlier, due to the plasmon wavelength of GNRs can enshroud the visible and NIR areas by adjusting aspect ratio, GNRs is an ideal candidate for GNVs [24-26]. For instance, Song [25] prepared a small GNRs (about 60 nm in size), which is assembled from small GNRs (about $8 \times 2$ nm). These GNRs use PEG and polyactic acid-glycolic acid (PLGA) coating and synthetized by emulsion method. Their Maximum absorption wavelengths are from 800 to 1050 nm. At the same optical density (OD) value, the PA intensity of the GNVs is about ten times higher than that of GNRs illuminated with 808 nm laser. Subsequently, based on Au@PEG-Fe$_3$O$_4$@PS, Au@PS-Fe$_3$O$_4$@PEG and Au-Fe$_3$O$_4$@PEG/PS, Song [27-28] prepared Double-Layered vesicles (DL-Ve 1 and DL-Ve 2) and Mono-Layered vesicles (ML-Ve 3), separately. Under the same OD$_{785}$, PA amplitude of DL-Ve 2 was 2.2 times and 5 times stronger than that of DL-Ve 1 and ML-Ve 3, respectively. Interestingly, on account of the Janus amphiphilic gold–iron (II,III) Oxide Nanoparticles as a core, they indicated the capacity to be used as a dual modal imaging (optical and magnetic resonance imaging) agent in vivo. They adopted the DL-Ve 2 as dual modal imaging agents to determine vesicle accumulation in the tumor area after intravenous injection of vesicles into PBS. The sustained improvement of the PA signals and strength over time in the tumor region confirmed the accumulation and distribution of the vesicle in the tumor [22]. Using high-resolution 3D PA images of the tumor, they obtained a lot of information about the tumor, such as location, size, and morphology.

Except the dual PA and MR imaging, in recent years, researchers explored various strategies to make GNVs have more imaging capabilities, rather than giving it its own physical and chemical characteristics, achieving multimodal imaging of tumors based on GNVs, such as PET/PA [25-26] dual modal imaging, and even FL/PT/PA trimodal imaging [29].
3. Conclusions and perspectives

In this review, we concentrated on the latest development of GNPs for PA imaging, due to their chemical/biological inertness in various cell and animal models, adjustable optical properties and low cytotoxicity. However, in order to make GNP have better capability in the field of biomedicine, and even turn to clinical translation, more efforts are still needed[22]. The first, researchers should take measures to improve the photostability of GNPs, during PA imaging some plasmonic GNPs can change their shapes[9]. Second, apply measures to produce strong PA imaging signals at relatively low doses of GNPs and multi-modality biomedical imaging by janus GNPs. Third, since a few endogenous biomolecules can absorb or emit NIR-II light, it is necessary to develop suitable contrast agents used for PA imaging in the NIR-II window[4].

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