Use of aerosol route to fabricate positively charged Au/Fe₃O₄ Janus nanoparticles as multifunctional nanoplatforms

Jeong Hoon Byeon¹ & Jae Hong Park²

Gold (Au)-decorated iron oxide (Fe₃O₄), Au/Fe₃O₄, Janus nanoparticles were fabricated via the continuous route for aerosol Au incorporation with Fe₃O₄ domains synthesized in an aqueous medium as multifunctional nanoplatforms. The fabricated nanoparticles were subsequently exposed to 185-nm UV light to generate positive charges on Au surfaces, and their activities were tested in computed tomography (CT) and magnetic resonance (MR) imaging, gene-delivery and photothermal therapy. No additional polymeric coatings of the Janus particles also had a unique ability to suppress inflammatory responses in macrophages challenged with lipopolysaccharide, which may be due to the absence of amine groups.

Heterogeneous nanostructures consisting of different nanoscale components that may introduce multifunctionality have received a great deal of attention in various applications, such as energy production/storage, catalysis, and biomedical applications. Because of the synergetic or complementary effects from their unique structural and interfacial properties, hetero-nanostructures can derive favorable physicochemical characteristics that can break through the current technical limits to the realistic employment of monocomponent nanomaterials for future nanotechnology¹. As one of the representative hetero-nanostructures, Janus or dumbbell-like particles consisting of different components have been considered favorably due to their multifunctionality². For biomedical applications, in particular, the use of hetero-nanostructures as probes via the incorporation of two or more functional materials may introduce dual- or multi-modal responses to enable therapeutics and diagnostics to be performed simultaneously³.

Gold (Au)-decorated iron oxide (Fe₃O₄), Au/Fe₃O₄ nanostructures have received great attention as representative hetero-nanostructures because of their biocompatibility and multifunctionality. The incorporation of Au and Fe₃O₄ components can basically bring together plasmonic and magnetic properties, and these components are representative biocompatible metallic components to be employed in biomedical applications⁴,⁵. Specifically, various Au nanostructures have recently been adopted in chemo- or photo-thermal therapies because of their surface plasmon resonance (SPR) heating when they are placed on visible or near-infrared (NIR) irradiation⁶–⁷. Particularly, NIR has high transmission efficiency into water or hemoglobin; thus it may be more suitable to penetrate into deep tissues to treat or kill cancer cells using a non-invasive method. The high thermal energies from the irradiation are capable of treating or killing cancer cells owing to thermoresponsive drug release and the hyperthermic effect⁷.

Numerous approaches have been proposed for synthesizing functional nanomaterials that mainly consist of Au and Fe₃O₄ nanoparticles⁸,⁹. More recently, gold-iron oxide hybrid nanomaterials have been further considered to create multimodal theranostic nanoplatforms for biomedical applications using multiple wet chemical processes¹⁰–¹². Template- or seed-mediated chemical approaches, where preformed metallic particles are used as templates or seeds for growing other metallic components on their surfaces, are well known for synthesizing hetero-nanostructures without forming monometallic particles. However, incorporating quantitatively different metallic components to form hetero-nanostructures is challenging, because most approaches consist of tedious multi-step wet chemical processes (which can lead to the generation of unwanted products), and this also requires additional cationic coating on the surfaces for targeting and/or binding purposes¹³,¹⁴. Moreover, to form metallic
hetero-nanostructures in a chemical bath, a reducing agent (mostly toxic) converts metal ions to metal atoms; however, this may introduce complexities to the reaction and separation, and its use may be restricted in biomedical applications. Despite the successful combination of the Au and Fe₃O₄ (or Fe₂O₃) components, the introduction of practical generalizable assembly strategies in a continuous manner is a huge challenge in the preparation of anti-inflammatory nanoplatforms without significant changes to the preparation system.

In this study, the potential use of positively charged Au/Fe₃O₄ Janus nanoparticles as multifunctional nanoplatforms via the single-pass aerosol route without multi-step chemical reactions and additional cationic coatings is discussed for use in computed tomography (CT)-magnetic resonance (MR) dual-mode imaging, gene-delivery, and photothermal therapy. Freshly produced aerosol Au nanoparticles were first injected into a collision atomizer filled with Fe₃O₄ nanoparticles to form hetero-droplets, and the droplets were successively injected into a diffusion dryer containing pelletized activated carbons and silica gels to extract solvent from the droplets, resulting in Au/Fe₃O₄ Janus nanoparticles. The Janus nanoparticles were then exposed to 185 nm UV radiation to eject some electrons from the surfaces of the nanoparticles in a single-pass configuration (Supplementary Fig. S1). Finally, the positively charged nanoparticles were electrostatically collected to evaluate their ability for CT-MR imaging, gene-delivery, and photothermal cancer cell killing [exposed to 705 nm wavelength laser light to inhibit adenosine triphosphate (ATP) production in cancer cells].

Results and Discussion

A gas temperature of approximately 6000 °C was generated between two cylindrical Au rods, and parts of the rods evaporated. The duration of spark formation was approximately 1 ms, and the evaporated Au nucleated right after the spark channel by N₂ gas flow, forming Au nanoparticles. The particle size distribution was analyzed using a scanning mobility particle sizer (3936, TSI, USA) to verify the concentration, mean diameter, and standard deviation. The measured concentration, diameter, and standard deviation of the Au particles were $3.45 \times 10^6$ particles cm⁻³, 19.7 nm, and 1.28, respectively, as shown in Fig. 1. Au/Fe₃O₄ hetero-structures were fabricated via floating self-assembly, where Au nanoparticles were attached on Fe₃O₄ domains through single-pass collision atomization and solvent extraction, which were evaluated by a comparison of the size distributions (i.e., Fe₃O₄ and Au/Fe₃O₄ configurations, summarized in Supplementary Table S1) in the aerosol state. The particle concentration, diameter, and standard deviation of the Au/Fe₃O₄ particles were $4.10 \times 10^6$ particles cm⁻³, 24.7 nm, and 1.49, respectively. Analogous data for the Fe₃O₄ particles were $3.22 \times 10^6$ cm⁻³, 20.2 nm, and 1.46, respectively.

Even though different size distributions were combined physically, there were no bi-mode (two individual peaks of Au and Fe₃O₄) characteristics in size distribution. The results showed only a new uni-mode size distribution and they were closer to those of the Au particles than those of the Fe₃O₄ domains, suggesting that the Au particles were attached well enough to the Fe₃O₄ domains to construct Au/Fe₃O₄ hetero-nanostructures.

The prepared particles (Au, Fe₃O₄, and Au/Fe₃O₄) in the aerosol state sampled on a carbon-coated copper grid were analyzed using a transmission electron microscope (TEM, JEM-3010, JEOL, Japan) (Fig. 2) to understand the formation of the Au/Fe₃O₄ hetero-nanostructures through the aerosol route. The particles were electrostatically deposited on the grid using a commercial aerosol collector (NPC-10, HCT, Korea). As shown in Fig. 2, the TEM observations suggest that the primary Au particles (about 3 nm mean diameter) were agglomerates, implying that the primary particles collided with each other after they formed near the spark. In the case of Fe₃O₄ domains, a square-like shape with a size of about 20 nm was observed, which was different from the spherical shape observed when Fe(CO)₅ was used as the Fe-precursor. For the Au/Fe₃O₄ configuration, a darker-contrast domain of spherical particles was exhibited in the Miller plane (111) owing to a 0.242-nm face-centered cubic Au lattice (inset), whereas lighter-contrast domains of the randomly deposited spherical particles were attributable to the Fe₃O₄ particles, showing the lattice spacing was about 0.485 nm (also see inset). Scanning electron microscope (SEM, NOVA nanoSEM, FEI, USA) images (Supplementary Fig. S2) further confirm the Au/Fe₃O₄ configuration. Thus, it was clearly observed that Au particles could be incorporated with Fe₃O₄ domains via floating
The incorporation of Au on Fe₃O₄ generated an absorption shift from the visible to NIR range, suggesting that the agglomerated Au particles were redistributed or reorganized as Janus nanoparticles via incorporation with Fe₃O₄ domains. X-ray diffraction (XRD) patterns of the Au, Fe₃O₄, and Au/Fe₃O₄ samples were analyzed. The six characteristic bands at 30.4° (220), 35.4° (311), 43.2° (400), 53.4° (422), 57.2° (511), and 62.7° (440) were found in the pure Fe₃O₄ sample. In the case of Au/Fe₃O₄, an additional peak was measured at 38.1° (111), suggesting that the floated Fe₃O₄ domains were physically incorporated with face-centered cubic Au particles (well matched with the profile of pure Au particles in Supplementary Fig. S4b) as homogeneous crystallites.

The formation of Au/Fe₃O₄ Janus nanoparticles was also verified by UV-vis absorption spectroscopy (Supplementary Fig. S4a). The Au nanoparticles showed an absorption peak at around 525 nm, whereas the spectrum of the Au/Fe₃O₄ showed that the incorporation of Au on Fe₃O₄ generated an absorption shift from the visible to NIR range, suggesting that the agglomerated Au particles were redistributed or reorganized as Janus nanoparticles via incorporation with Fe₃O₄ domains. X-ray diffraction (XRD) patterns of the Au, Fe₃O₄, and Au/Fe₃O₄ samples were analyzed. The six characteristic bands at 30.4° (220), 35.4° (311), 43.2° (400), 53.4° (422), 57.2° (511), and 62.7° (440) were found in the pure Fe₃O₄ sample. In the case of Au/Fe₃O₄, an additional peak was measured at 38.1° (111), suggesting that the floated Fe₃O₄ domains were physically incorporated with face-centered cubic Au particles (well matched with the profile of pure Au particles in Supplementary Fig. S4b) as homogeneous crystallites.

To employ the Janus nanoparticles in MR imaging, the particles were placed in a vibrating sample magnetometer to characterize the magnetic properties at 300 K. Supplementary Fig. S5 shows the paramagnetic characteristics without hysteresis of Fe₃O₄ for both the Fe₃O₄ and Au/Fe₃O₄ samples, corresponding to free coercivity (or remanence). After the magnetic properties were measured, the abilities of the Au/Fe₃O₄ Janus nanoparticles for CT-MR dual-mode imaging of a phantom were measured at different mass concentrations (mg mL⁻¹) for CT and concentrations (mM for MR) after the sampled aerosol particles were suspended in water. For measurements of CT contrast ability, the signal intensity was proportional to the particle concentration (inset of Supplementary Fig. S5), which implies that the prepared Au/Fe₃O₄ would be available for X-ray imaging applications. To examine MR imaging, an in vitro T₁-weighted MR imaging experiment was further conducted at the same phantom. In the case of MR imaging, the signal intensity was inversely proportional to the particle concentration (with an r² of 130.4 mM⁻¹ s⁻¹, inset of Supplementary Fig. S5). Since the Janus particles are sensitive in both CT and MR imaging, the nanoparticles are expected to have potential for application in CT-MR dual-mode imaging.

The sampled nanoparticles on a glass substrate were detached in an ultrasound bath to secure stability for a long-term storage. The dynamic light scattering (DLS) (Nano ZS90, Malvern Instruments, UK) measurements of the photoionized Au/Fe₃O₄ particles were additionally performed. The directly gas-phase sampled particles on a glass plate were applied just before [i.e., the injection of the particles on a glass plate in phosphate buffered saline (PBS) solution was performed just before DLS measurements and biological assessments] required assessments. The results showed that the deviation of hydrodynamic diameter is no larger than 6.1% for the particles, and there are no significant differences between the storage days (1–14 days). This implies that the particles have stability that warrants further investigation. The cytotoxicities of the aerosol-fabricated Au/Fe₃O₄ nanoparticles, including individual Au and Fe₃O₄ nanoparticles, were tested to verify their biocompatibility using a MTS assay. As shown in Fig. 3a, the cell viabilities were over 96% for the Au/Fe₃O₄ and photoionized Au/Fe₃O₄ samples at different concentrations from 10 to 90 μg mL⁻¹, whereas the viabilities for the Au and Fe₃O₄ particles were over 97% and 85%, respectively. To further evaluate the biocompatibility of photoionized Au/Fe₃O₄ sample (Fig. 3b), two specific dyes, 4′,6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, US) and propidium iodide (PI) (BD Science, US), were employed to verify viability of cells with the sample. DAPI (blue-350 nm) stains both live and dead cells, whereas PI (red-490 nm) stains only the dead cells, allowing for selective visualization.
dead cells, while PI (red-488 nm) may only pass through the membranes of dead cells. By examining the fluorescence (IX71, Olympus, US), cell viability was then confirmed, and the results supported the MTS assay that the photoionization did not increase the cytotoxicity of the Au/Fe3O4 particles. In particular, the cytotoxicity of the Janus particles did not show significant differences compared with individual Au particles, and this implies that the incorporation of Au with Fe3O4 may even reduce the slightly higher cytotoxicity of the Fe3O4 nanoparticles. Specifically, enzymatic degradation in cells via catalytic oxidative stress from Fe3O4 nanoparticles might be somewhat prevented by the presence of the Au particles on the Fe3O4 domains. This suggests that the Janus particles are not cytotoxic and are likely suitable as nanoplatforms for biomedical applications. To determine the gene-delivery ability of the photoionized Janus particles, the particles were mixed with plasmid DNA to form Au/Fe3O4-gene complexes and placed in 293 human embryonic kidney cells. The untreated Janus particles showed a higher gene-delivery efficiency than that of naked DNA, and the efficiency was significantly increased when the Janus particles were photoionized, even higher than that of polyethylenimine (PEI)-gene complexes (Fig. 3c). Figure 3c (inset) also shows a visualized enhanced green fluorescent protein (EGFP) distribution in the cells for the photoionized Janus particles, and this provides further evidence of the enhanced gene-delivery via photoionization. In the present work, direct photoionization was employed to create positively charged Au/Fe3O4 particles, since the photon energy of the employed UV (6.22 eV) is higher than the work function (5.31 eV) of the Au particles that induced electron ejections when the photons absorbed on an Au particle. The enhanced efficiency of the photoionized Janus particles may have been due to the positive charges on the particle surfaces. To verify the electron ejections from the nanoparticles (i.e., positive charges on the particles), we measured the charge distributions of the nanoparticles after the UV irradiation using a tandem differential mobility analyzer (TDMA) system. Figure 3d shows the charge distributions of the particles exposed to UV light with a wavelength of 185-nm. The different peaks appearing in the image represent the different numbers of positive charges on the Janus particles. The ratio between the measured mobilities of two types of nano DMA (NDMA, 3085, TSI, USA) corresponds to the number of charges (q) of the photoionized Janus particles, and it is given by

$$q = \frac{Z_{p, DMA2}}{Z_{p, DMA1}}$$

(2)

where $Z_{p, DMA1}$ and $Z_{p, DMA2}$ are the measured mobilities using NDMA 1 and 2, respectively. According to the results, the Janus particles were positively charged upon photoionization, and this proves that electrons were ejected from the nanoparticles. The result reveals that the UV irradiation induced positive charges on the particles and that the mean number of charges for the Au/Fe3O4 particles was 1.44. This also introduced a positive value (+3.4 mV) in zeta potential (measured also using Nano ZS90, Malvern Instruments, UK) of the particles in PBS solution. Supplementary Fig. S6 shows the Au 4f X-ray photoelectron spectroscopy (XPS) spectrum of UV-exposed Au/Fe3O4 nanoparticles. Interestingly, the spectrum also shows a new feature compared with the bulk Au. A well-defined peak appears at 85.2 eV, which is assigned to ionized Au species (Au$^{+}$), corresponding
to the positive charges measured by the TDMA system, whereas there is no additional peak in the untreated Au/Fe₃O₄ nanoparticles. Quantitative cellular uptake of photoionized Au/Fe₃O₄ nanoparticles (Supplementary Fig. S7) was performed using a fluorescence-activated cell sorting (FACS, BD Biosciences, USA). In the cells, the photoionized particles incorporated with fluorescein isothiocyanate (FITC, as fluorescent tracers, shown in inset of Supplementary Fig. S7) were uptaken in a time-dependent manner. These results suggested further investigation of the photoionized particles.

Au/Fe₃O₄ hetero-nanostructures have recently been explored as photoinducers for photothermal cancer cell killing under NIR irradiation⁴. To verify this ability, the Janus nanoparticles were injected into agar gel (as a simulated physiological condition), and they were exposed to a laser with a 705-nm wavelength (Fig. 4a). According to the absorption spectrum (Supplementary Fig. S4a), the laser was selected as the NIR irradiation source to generate heat. The temperature changes under the laser irradiation for durations of 10, 30, and 60 s were measured using an IR thermometer (42545, Extech, USA). In the absence of the Janus particles, no temperature change (ΔT, Supporting Information) was found for the same irradiation durations. The temperatures of the agars gels were significantly changed for 10, 30, and 60 s irradiations when the gels contained 10, 30, 50, 70, and 90 μg mL⁻¹ of Janus nanoparticles (Fig. 4b). The ΔT value was proportional to the particle concentrations, and the value reached 40.3 °C at the highest concentration (90 μg mL⁻¹) and longest irradiation time (60 s). From the temperature measurements, it can be concluded that the Janus nanoparticles absorbed the irradiated laser light, and the light was successively converted into thermal energy.

To measure the photothermal activity of the particles for cell killing, an adenosine triphosphate (ATP) assay was employed, since ATP production is related to glycolysis for most cancer cells²⁴. A375M cell lines were used to

**Figure 4.** Results for photothermal therapy using Au/Fe₃O₄ Janus nanoparticles under 705 nm wavelength laser irradiation. (a) Scheme of evaluation process using *in vitro* ATP assay for photothermal therapy. (b) Temperature change in Au/Fe₃O₄ nanoparticles at different particle concentrations (10–90 μg mL⁻¹) by irradiation of 705-nm wavelength laser for durations of 10, 30, and 60 s. (c) Photothermal activity to reduce ATP levels for different laser irradiation times (10–60 s) on Au/Fe₃O₄ nanoparticles at particle concentrations of 10, 30, 50, 70, and 90 μg mL⁻¹.

**Figure 5.** MIP production from LPS-challenged macrophages by adding photoionized and PEI, PLL, and PEG incorporated (2 × 10⁻⁶ mol dm⁻³) Janus particles. Insets show representative TEM images (scale bar, 20 nm) of the polymer incorporated Janus particles.
test whether laser exposure can affect ATP production. According to optical microscopy observations, the Janus particles (dark spots, inset of Fig. 4c) were located mostly inside the cell or on the surface of the cell, which confirms the particles’ presence in the cell. No temperature increase in the absence of the Janus particles introduced no significant changes in ATP production, whereas the thermal energy in the presence of the particles obstructed the ATP production of the cells owing to the heat created when the laser irradiated the agar gels. The decrease in ATP production level may have been due to stress gene overexpression (i.e., inducing increasing demands on the ATP cellular pool) via the hyperthermic effect under laser irradiation in the presence of the Janus particles 

Furthermore, another scenario for parenteral applications was considered, where the Janus particles are administered to tissues that attract activated macrophages. In order to suppress inflammatory responses, nanoparticle interaction with biological system has recently been studied for nanoparticulate delivery systems 

The results (Fig. 5) show that the (bio)photoionized Janus particles could more significantly suppress the macrophage inflammatory protein (MIP) production from lipopolysaccharide (LPS)-challenged macrophages than those from polyethyleneimine (PEI) or poly-l-lysine (PLL) incorporated Janus particles (insets of Fig. 5). The smaller MIP productions of the photoionized Janus particles than that from the PEI or PLL incorporated particles indicate that the tendency may be related to the amine content. A similar result from the polyethylene glycol (PEG) incorporated Janus particles (another inset of Fig. 5) further confirms this hypothesis because of no amine groups in PEG. 

This study discussed the use of the aerosol route for the fabrication of positively charged Au/Fe3O4 Janus nanoparticles via the floating self-assembly of Au and Fe3O4 components and successive photoionization to liberate several electrons from the Janus particles. The fabricated Janus nanoparticles with positive charges were then employed to confirm their biocompatibility and multifunctional abilities for CT-MR dual-mode imaging, gene-delivery, and photothermal therapy without significant increases in cytotoxicity or changes in morphology. Even though all previous Au-based hetero-nanostructures employed polymeric or macromolecular organic layers on which nanostructures were loaded, this study suggests a new possibility of fabricating multifunctional nanoplastforms with the minimized inflammatory responses without the use of multiple wet chemical processes and organic compounds. This original, fundamental strategy of unusual urgency and significance may appeal to a broad, general audience in biomedical applications.

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Author Contributions
J.H.B. and J.H.P. conceived the project, and designed and performed the experiments, and wrote the manuscript.

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