Development of bimetallic (Zn@Au) nanoparticles as potential PET-imageable radiosensitizers

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Purpose: Gold nanoparticles (GNPs) are being investigated actively for various applications in cancer diagnosis and therapy. As an effort to improve the imaging of GNPs *in vivo*, the authors developed bimetallic hybrid Zn@Au NPs with zinc cores and gold shells, aiming to render them *in vivo* visibility through positron emission tomography (PET) after the proton activation of the zinc core as well as capability to induce radiosensitization through the secondary electrons produced from the gold shell when irradiated by various radiation sources.

Methods: Nearly spherical zinc NPs (~5-nm diameter) were synthesized and then coated with a ~4.25-nm gold layer to make Zn@Au NPs (~13.5-nm total diameter). 28.6 mg of these Zn@Au NPs was deposited (~100 μm thick) on a thin cellulose target and placed in an aluminum target holder and subsequently irradiated with 14.15-MeV protons from a GE PETtrace cyclotron with 5- μA current for 5 min. After irradiation, the cellulose matrix with the NPs was placed in a dose calibrator to assess the induced radioactivity. The same procedure was repeated with 8-MeV protons. Gamma ray spectroscopy using an high-purity germanium detector was conducted on a very small fraction (<1 mg) of the irradiated NPs for each proton energy. In addition to experimental measurements, Monte Carlo simulations were also performed with radioactive Zn@Au NPs and solid GNPs of the same size irradiated with 160-MeV protons and 250-kVp x-rays.

Results: The authors measured 168 μCi of activity 32 min after the end of bombardment for the 14.15-MeV proton energy sample using the ^{66}Ga setting on a dose calibrator; activity decreased to 2 μCi over a 24-h period. For the 8-MeV proton energy sample, PET imaging was additionally performed for 5 min after a 12-h delay. A 12-h gamma ray spectrum showed strong peaks at 511 keV (2.05×10^6 counts) with several other peaks of smaller magnitude for each proton energy sample. PET imaging showed strong PET signals from mostly decaying ^{66}Ga . The Monte Carlo results showed that radioactive Zn@Au NPs and solid GNPs provided similar characteristics in terms of their secondary electron spectra when irradiated.

Conclusions: The Zn@Au NPs developed in this investigation have the potential to be used as PET-imageable radiosensitizers for radiotherapy applications as well as PET tracers for molecular imaging applications. © 2016 American Association of Physicists in Medicine. [<http://dx.doi.org/10.1118/1.4958961>]

Key words: bimetallic nanoparticle, hybrid nanoparticle, Zn@Au nanoparticle, gold nanoparticle (GNP), medical cyclotron, proton activation, positron emission tomography (PET)

1. INTRODUCTION

Lately, gold nanoparticles (GNPs) have been actively investigated for various applications in cancer diagnosis and therapy. For example, many computational and experimental studies have been performed to develop GNPs into dose-enhancing or

radiosensitizing agents for radiation therapy.^{1–25} When GNPs are bombarded by radiotherapy beams, cascades of secondary particles such as photoelectrons and Auger electrons are generated from GNPs as a result of interactions between GNPs and radiation (e.g., photons, electrons, and protons). Due to their short ranges (on the order of micrometers or even less)

in tissue-like media, these secondary particles deposit their energy locally and, when GNPs are located in close proximity to the cell nucleus, the localized energy deposition (or particle tracks) can result in DNA damage via direct (breakage of DNA strands) or indirect (generation of reactive oxygen species) effects, leading to cell death.⁶

In addition to research effort to fully elucidate the underlying physical, chemical, and biological mechanisms of GNP-mediated radiosensitization, the development of a noninvasive imaging tool for monitoring the distribution of GNPs *in vivo* is also critical for a successful translation of GNP-mediated radiosensitization strategies into clinical practices. In recent years, several imaging modalities, such as x-ray fluorescence imaging or computed tomography (XFCT)^{26–35} and photoacoustic imaging,^{36–41} have been suggested for this purpose and are currently under active development. While promising, these approaches need to be developed further, especially in terms of their applicability to human imaging applications. Some researchers have also studied the technique of directly labeling GNPs with radioisotopes that can be imaged using single photon emission computed tomography (SPECT).^{42,43} Despite many advantages, this method requires binding linkers in order to externally radiolabel GNPs [on top of already existing coating such as polyethylene-glycol (PEG)] which has the concern for possible detachment of radioisotopes resulting in erroneous estimation of GNP distributions.^{44,45}

Recently, some investigators directly activated various NPs using protons or neutrons to make radioactive nanoparticles.^{46–48} These NPs decay while emitting gamma rays or positrons and therefore can be imaged using SPECT or positron emission tomography (PET). Among such innovative research, Pérez-Campaña *et al.* developed ¹⁸O-enriched titanium oxide NPs (TiO₂ NPs). When activated with protons, ¹⁸O in NPs became ¹⁸F, which decayed with positron emission and could be imaged using a PET scanner. When injected into mice, TiO₂ NPs showed an excellent time-dependent biodistribution; however, owing to the relatively short decay half-life of ¹⁸F (109 min), *in vivo* imaging was obtainable only up to 8 h after injection.⁴⁹ In addition, TiO₂ NPs are likely less effective than GNPs for radiosensitization, due to relatively smaller atomic numbers of TiO₂ (23 for titanium and 6 for oxygen, compared with 79 for gold).

Although GNPs can be directly activated by protons, the positron emission yield of activated GNPs is extremely low compared with other gamma rays; therefore, SPECT appears to be a more viable method than PET for *in vivo* imaging even though its sensitivity is several orders of magnitude lower than PET.⁵⁰ Therefore, we sought to develop hybrid bimetallic NPs consisting of zinc and gold (Zn@Au) which may offer similar dosimetric characteristics as GNPs (made of 100% gold) for the purpose of radiosensitization, while imageable using highly sensitive PET. This technique could be potentially applicable to typical radiation treatments for deep-seated human tumors within the context of GNP-aided radiotherapy. Moreover, PET-visible GNPs provide a new option for molecular imaging when combined with various tumor-targeting approaches.

FIG. 1. (a) Configuration of synthesized gold-coated zinc (Zn@Au) NPs. This model assumed a nominal diameter/thickness (5/4.25 nm) of zinc core and gold shell. (b) When irradiated with protons, the zinc core is activated to generate positron emitters (⁶⁴Ga, ⁶⁶Ga, and ⁶⁸Ga). Note the volume and mass fractions (in %) of Au and Zn in each Zn@Au NP are 94.9% and 5.1%, and 98.1% and 1.9%, respectively.

2. MATERIALS AND METHODS

Zn@Au NPs were developed to take advantage of the unique properties of both zinc and gold (Fig. 1). When irradiated with protons, natural zinc cores are activated to create the positron emitters ⁶⁴Ga (*T*_{1/2} = 2.63 min), ⁶⁶Ga (*T*_{1/2} = 9.50 h), and ⁶⁸Ga (*T*_{1/2} = 67.72 min) from naturally existing stable zinc isotopes (Fig. 2). The gold shells are expected to provide the secondary electrons necessary for radiosensitization.

2.A. Synthesis and characterization of Zn@Au nanoparticles

The synthesis of Zn@Au NP of approximately 10–20 nm in diameter was sought in the current proof-of-principle study as similarly sized GNPs were previously tested for radiosensitization.^{3,12,13} For the NP synthesis, the diameter of Zn core was chosen such that it was approximately the same as the Au shell thickness, in order to ensure a reasonable balance between PET signals from the Zn core and the secondary electrons (for radiosensitization) from the Au shell. Zinc(II) chloride (ZnCl₂), poly(vinylpyrrolidone) (PVP; molecular

FIG. 2. Nuclear interaction cross sections leading to radioactive gamma ray, positron, and electron emissions for natural zinc and gold bombarded with low-energy protons (data obtained from Brookhaven National Laboratory EXFOR library). When bombarded with protons, ⁶⁸Zn, ⁶⁶Zn, and ⁶⁴Zn are activated to create ⁶⁸Ga, ⁶⁶Ga, and ⁶⁴Ga, which decay via positron emission with high-branching ratios (B+) of 90%, 57%, and 98%. The natural abundance of ⁶⁸Zn is 18.8%; for ⁶⁶Zn, 27.9%; and for ⁶⁴Zn, 48.6%. One hundred percent of natural gold exists as ¹⁹⁷Au. Two vertical lines represent the proton energies (14.15 and 8 MeV) used to bombard gold-coated zinc (Zn@Au) NPs. The nuclear interaction energy threshold of gold occurs at ~8 MeV.

weight 10 000), lithium borohydride (LiBH_4), mesitylene, chlorauric acid (HAuCl_4), sodium borohydride (NaBH_4), and sodium citrate were purchased from Sigma-Aldrich (St. Louis, MO), and all chemicals and solvents were used as received.

First, approximately spherical zinc NPs (~5-nm diameter) were synthesized according to the procedure published by Ghanta *et al.*⁵¹ Briefly, the reactants ZnCl_2 (0.136 g, 1.0 mmol), LiBH_4 (0.044 g, 2.0 mmol), and PVP (the stabilizer, 55.5 mg) were added to mesitylene (20 ml) in a 100-ml, 2-necked, round-bottom flask equipped with a magnetic stirring bar, a reflux condenser, and a nitrogen inlet/outlet. The reaction mixture was refluxed for 24 h under static nitrogen atmosphere under stirring. Upon completion of the reaction, a quick work-up was processed to avoid the oxidation of the zinc NPs; the mixture was cooled to room temperature and centrifuged to collect the solids, which were then washed with 20–30-ml portions of ice-cold dry methanol to remove the lithium chloride and other side products of the reaction. The black-grey colored residue was dried under a vacuum and characterized as zinc NPs (yield: 82%). The zinc NPs were stored in a vacuum desiccator.

Second, the synthesized zinc NPs were coated with a ~4.25-nm thick gold layer (for a total diameter of ~13.5 nm) using the reactants HAuCl_4 , NaBH_4 (the reductant), and sodium citrate (the stabilizer) in deionized water under static nitrogen atmosphere. Next, 64 mg of zinc NPs, 22 mg of sodium citrate, and 95 mg of NaBH_4 were added to 100 ml of deionized water under static nitrogen atmosphere and cooled to 0 °C. Under vigorous stirring, a HAuCl_4 solution (2.5 mmol) was added dropwise, and the black suspension gradually came out. After the reaction, the solid was collected by centrifuge and washed with water several times. Finally, sodium 3-mercapto-1-propanesulfonate was applied to exchange the original stabilizers to make the synthesized Zn@Au NPs more stable and water-compatible.

The core-shell morphology, size, and layer-thickness of Zn@Au NPs were investigated by transmission electron microscopic (TEM) measurements using the average particle size of the parent Zn NPs as determined from its micrograph serving as a reference for comparison. Ultraviolet-visible spectroscopy (UV-vis spectra) was also performed for Zn NPs and Zn@Au NPs in water to detect absorption spectra in the near-ultraviolet and visible spectral region.

2.B. Proton activation, gamma ray counting, and PET scan of Zn@Au nanoparticles

A water slurry containing 28.6 mg of Zn@Au NPs was deposited in layers up to approximately 100 μm thick on a thin cellulose target and subsequently baked to remove the water. The cellulose matrix was placed in an aluminum target holder and irradiated with 14.15-MeV protons (after passing through the 50- μm aluminum target window) from a GE PETtrace cyclotron (GE Healthcare, Waukesha, WI) with 5- μA current for 5 min. The cellulose matrix containing the Zn@Au NPs was placed in a Capintec dose calibrator (CAPINTEC,

Inc., Ramsey, NJ) and radioactivity was assessed starting 32 min after the end of bombardment (EOB), continuing over a period of 24 h. We used the ^{66}Ga setting on the dose calibrator because ^{66}Ga ($T_{1/2} = 9.50$ h) was the only source of radioactivity several hours after EOB (after ignoring ^{197}Hg whose contribution is insignificant for the period of measurement).

A very small fraction (<1 mg) of the irradiated Zn@Au NPs (without cellulose matrix) were moved to a clean (unirradiated) vial and placed in a nitrogen-cooled, high-purity germanium (HPGe) detector (ORTEC, Oak Ridge, TN), and gamma ray spectroscopy was started 2 h after EOB, continuing over a period of 12 h. Owing to the high sensitivity of the HPGe detector, greater than 90% dead time was recorded when the vial was placed in the center of the detector. Therefore, the vial was placed ~20 cm away from the center (on top of a lead shield with a small opening to the detector) and less than 3% dead time was recorded throughout the measurement. A second round of gamma ray spectroscopy using the HPGe detector was started at 70 h after EOB, continuing for 12 h. This time, the entire irradiated target of Zn@Au NPs (28.6 mg), including the cellulose matrix, was used.

The above measurement was repeated using 8-MeV protons. A 23-mg Zn@Au NP sample was placed on a 0.76-mm thick aluminum sheet and taped over with high-temperature aluminum tape with fiberglass and silicone adhesive (CS Hyde Company, Lake Villa, IL), which maintained its hold for temperatures of up to 500 °F. The target (aluminum sheet+Zn@Au NPs+tape) was placed in the target holder with the aluminum sheet toward the beam's upstream direction. The incident energy of the proton beam degraded after the beam passed through the 0.76-mm thick aluminum sheet and bombarded the Zn@Au NPs with 8-MeV protons. The target was irradiated with 5- μA proton current (using the GE PETtrace cyclotron) for 5 min. After irradiation, the target was placed in the Capintec dose calibrator and radioactivity was assessed starting 17 min after EOB, continuing over a period of 5 h. A very small fraction (<1 mg) of Zn@Au NPs was placed in the HPGe detector for gamma ray spectroscopy in a similar manner as described above, beginning 4.5 h after EOB and continuing for a period of 1.5 h.

Following the radioactivity assay and gamma ray spectroscopy, the original target (bombarded with 8-MeV protons) used for the radioactivity assay was moved to a PET/CT scanner (Discovery PET/CT 690 scanner, GE Healthcare, Waukesha, WI) for imaging. The PET scan started at 12 h after EOB and the image was reconstructed for 5-min acquisition.

The purpose of using 2 different proton energies was, first, to take advantage of the high proton-zinc interaction integral cross section, which led to high yield for ^{66}Ga and ^{68}Ga radioisotopes using the higher incident energy (14.15 MeV; Fig. 2). However, this had the disadvantage of creating undesired ^{197}Hg isotopes from gold activation. Thus, the lower incident proton energy, 8 MeV, was used to provide a lower yield owing to the lower integral cross section; this had the advantage of not activating gold owing to the relatively high interaction energy threshold of gold

(Fig. 2). For both 14.15 and 8-MeV proton activations, aluminum was not activated owing to its high interaction energy threshold (EXFOR library, Brookhaven National Laboratory Experimental Nuclear Reaction Data). However, oxygen (^{16}O) in cellulose, as well as in fiberglass and silicone (in the tape), has a threshold energy of about 6 MeV for the $^{16}\text{O}(p,\alpha)^{13}\text{N}$ interaction; therefore, contamination activation was expected.

The measured activity was compared with the calculated activity for the zinc cores only (assuming no gold activation) in the Zn@Au NPs. When zinc cores of the volume fraction V_f in Zn@Au NPs (in this case, 5.1%) with T atoms (in this case, ^{66}Zn and ^{68}Zn) and density ρ (7.14 g/cm³) are irradiated using a proton current of I , each resulting radioisotope r (in this case, ^{66}Ga and ^{68}Ga) is created according to its isotope production cross section $\sigma_{Tr}(E)$ (in this case, $\sigma[^{66}\text{Zn}(p,n)^{66}\text{Ga}]$ and $\sigma[^{68}\text{Zn}(p,n)^{68}\text{Ga}]$) divided by the stopping power of Zn@Au samples (dE/dx) over the proton energy E_i (initial energy

bombarding the top layer of Zn@Au NP samples) and E_f (final energy bombarding the bottom layer of Zn@Au NP samples), while simultaneously decaying according to its specific half-life. For simplification, because gold comprised 98.1% of the Zn@Au NP mass, the mass stopping power of gold (NIST PSTAR, Stopping Power and Range Tables for Protons) was multiplied by the density of Zn@Au NPs so that it could be used as the stopping power of Zn@Au samples. The accuracy of the simplified calculation was checked using the true composition of Zn@Au NPs via the stopping and range of ions in matter (SRIM) software (SRIM-2008.04). The SRIM-calculated stopping powers agreed with the simplified calculation within $0.2\% \pm 0.1\%$ up to the energy of 10 MeV which is the maximum energy capacity of the software. After irradiation, the activity or yield from the remaining radioisotopes at time t ($t = 0$ at EOB) can be presented as follows. Similar activity yield formulations can be found in several Refs. 52–54,

$$\text{Activity or Yield (when } t = 0 \text{ at EOB)} = I \cdot V_f \cdot \frac{N \cdot \rho}{M} \sum_T \int_{E_f}^{E_i} \frac{\sigma_{Tr}(E)}{\left(\frac{dE}{dx}\right)} dE \cdot A_T \cdot (1 - e^{-\lambda_r t_R}) e^{-\lambda_r t}, \quad (1)$$

where N is the Avogadro's number, M is the atomic weight of zinc, A_T is the natural abundance of each atom, λ_r is the radioisotope decay constant, and t_R is irradiation (beam-on) time. $V_f \rho$ represents the fractional density of zinc cores (m_{Zn}/V), which is the mass of zinc cores divided by the volume of the entire Zn@Au NP sample and also can be presented as $m_f \rho_{\text{Zn@Au}}$, which is the product of the mass fraction of zinc cores and the density of Zn@Au NPs. Table I shows the detailed measurement setups.

2.C. Monte Carlo (MC) studies

The first set of MC studies was performed to verify the nominal proton energy (14.15 and 8 MeV) used for activation studies. According to the specification of GE PETtrace, the proton beam is accelerated to 16.5 ± 0.1 MeV; after passing through 0.3 mm thick aluminum exit foil, the proton energy degrades to $14.5 \text{ MeV} \pm \Delta E$. This further degrades to $14.15 \pm \Delta E'$ or $8.00 \pm \Delta E''$ after passing through 0.05 mm or

TABLE I. Gold-coated zinc (Zn@Au) NP irradiation and measurement setups used in the current study. Note that the standard deviation at 1 σ level is denoted as “Stdev” in this table.

Measurement setup	First measurement (14.15 MeV)	Second measurement (8.00 MeV)
Target (Zn@Au NP) mass (mg)	28.6	23
Target diameter (mm)	8	8
Zn@Au NP density ($\rho_{\text{Zn@Au}}$) (g/cm ³)	18.85	18.85
Target area density (g/cm ²)	0.057	0.046
Target preparation method	Deposit on a cellulose	Tape over the dry sample
Beam current (μA)	5	5
Beam duration (min)	5	5
Beam energy on top of target (MeV) (Mean $\pm$ Stdev according to MC study) (MeV)	14.15 (14.24 $\pm$ 0.14)	8.00 (7.86 $\pm$ 0.33)
Beam energy on bottom of target (MeV) (Mean $\pm$ Stdev according to MC study) (MeV)	13.28 (13.39 $\pm$ 0.16)	6.95 (6.84 $\pm$ 0.38)
Aluminum activation	No	No
Gold activation	Yes	No
Contamination activation	Cellulose and gold activation	Silicone (in tape) activation
Positron emission tomography scan	No	Yes

Fig. 3. Setup for the second Monte Carlo simulations. The incident proton or x-ray beam interacted with nonradioactive gold-coated zinc (Zn@Au) NPs or GNPs placed in vacuum, as well as with the secondary particles. All secondary particles scored at the surface of NP.

0.05 + 0.75 mm thick aluminum entrance foils to Zn@Au NP samples. Monte Carlo simulation was performed to confirm the analytically calculated proton energies at the top and the bottom of the targets.

The second set of MC studies was performed to investigate possible difference between GNPs and Zn@Au NPs in terms of their secondary electron spectra resulting from external irradiation. Two radiation sources (160-MeV protons and 250-kVp x-rays) were used to bombard GNPs and Zn@Au NPs. A modulated spread-out Bragg peak proton beam delivered by a double-scattering system was simulated and validated⁵⁵ using the Tool for Particle Simulation (TOPAS) MC code,⁵⁶ with the beam line model based on The University of Texas MD Anderson Cancer Center proton gantry and nozzle blueprints provided by the manufacturer (Hitachi Ltd., Tokyo, Japan). A 160-MeV proton beam (10-cm spread-out Bragg peak; field size $18 \times 18 \text{ cm}^2$; range 11 cm) was simulated to irradiate a large water phantom ($30 \times 30 \times 15 \text{ cm}^3$), and the phase space file (for both primary and secondary particles) was recorded at a depth of 3 cm. The X and Y dimension of the phase space file was shrunk 5×10^6 -fold in each dimension ($18 \times 18 \text{ cm}^2$ – $36 \times 36 \text{ nm}^2$) to increase the chance for proton-nanoparticle interactions. More details about the methodology used for the current MC simulations can be found elsewhere.⁵⁷

Two types of NPs were simulated: first, a spherical Zn@Au NP was created with a 5-nm zinc core and a 4.25-nm gold shell, which made a Zn@Au NP with an outer diameter of 13.5 nm (Fig. 1); second, a GNP of the same diameter (13.5 nm) was created using solid gold. Both NPs were placed in a vacuum and bombarded with a proton beam of $36 \times 36 \text{ nm}^2$ incident from the beam origin located 50 nm upstream from the center of each NP (Fig. 3). The proton field was larger than the size of NPs to accommodate the interaction of nonparallel particles incident on the NPs. All NP-originated secondary particles escaping the NP were recorded on the outer surface of each NP.

For the x-ray simulation, a 250-kVp x-ray beam spectrum was generated using SpekCalc software^{58–60} with the following setup: 2-mm beryllium window, 0.1-mm inherent +0.25-mm copper filter, $N_f = 0.68$, $P = 0.33$, and $\theta = 30^\circ$. With the generated x-ray spectrum, a parallel x-ray beam

was generated to interact with the Zn@Au NP and GNP, and all secondary particles were recorded in a similar manner as described above. Figure 3 shows a simplified diagram for the first set of MC simulations.

The third set of MC studies was conducted to calculate the photon and electron spectra emitted by radioactive Zn@Au NPs. For simplicity, it was assumed that only the Zn cores were activated (for the case of 8 MeV proton bombardment) with the remaining activity solely from ^{66}Ga atoms (for the case in which ^{64}Ga and ^{68}Ga atoms decayed out). All source photon (including 511 keV photons from positron emissions) and electron emission spectra (with branching ratios $>0.3\%$) from decaying ^{66}Ga atoms were sampled (Brookhaven National Laboratory National Nuclear Data Center Chart of Nuclides). Three millions of photons and electrons each with the corresponding source energy spectra and branching ratios were randomly distributed inside the Zn core and set in motion with random directions. Both the primary and secondary particles escaping the NPs were scored at the surface of the NPs to estimate the attenuation and spectral changes (or energy degradation). Figure 4 shows schematics for the second set of MC simulations.

Fig. 4. Setup for the third Monte Carlo simulations. ^{66}Ga atoms that decay with positron, electron, and photon emission were randomly distributed inside the Zn core of a Zn@Au NP. The source photons and electrons from decaying ^{66}Ga radioisotopes were anticipated to attenuate and degrade in energy while escaping the Zn@Au NP.

FIG. 5. Transmission electron microscopy images and particle diameter histograms of (a) zinc NPs and (b) gold-coated zinc (Zn@Au) NPs. (c) UV-vis spectra of Zn NPs and Zn@Au NPs in water.

3. RESULTS

Figure 5(a) shows the TEM image of Zn NPs used in this study and their size distribution (average diameter $\pm 1\sigma$ standard deviation: 5.0 ± 1.5 nm). Subsequent chemical reduction of HAuCl_4 to cause the deposition of a gold layer onto the surface of Zn NPs resulted in Zn@Au NPs with the increased diameter of 13.5 ± 2.7 nm [Fig. 5(b)]. Histograms in Figs. 5(a) and 5(b) and the average NP diameters were calculated using 67 and 55 measurements with Zn NPs and Zn@Au NPs, respectively. By subtracting two average diameters, the average gold shell thickness was determined as 4.25 ± 3.1 nm. Figure 5(c) shows the UV-vis absorption spectra obtained from Zn NPs and Zn@Au NPs in water. Although no specific absorption peak is expected (or seldom reported) for Zn NPs, an exciton peak can be seen in the range of 360–380 nm for the oxidized Zn NPs (i.e., ZnO NPs).^{61–63} The absence of the 360–380 nm peak in the dotted curve in Fig. 5(c)

suggests that very little or no oxidation occurred during the synthesis and storage of Zn NPs. After the deposition of gold layer, the NP sample showed a classic gold plasmon resonance peak of 550 nm^{61,64} in the solid curve in Fig. 5(c), which indicates the existence of nanogold (i.e., Zn@Au NPs).

When Zn@Au NPs were irradiated with 14.15-MeV incident protons, we measured 168 ± 1 μCi of activity at 32 min after EOB, but this decreased to 2 ± 1 μCi over a 24-h period. Figure 6 shows the time activity curve of the measured activity, which is least-squares fitted with 3 simple exponential decay curves of ^{66}Ga , ^{68}Ga , and ^{13}N . With the initial activity of ^{66}Ga (8%), ^{68}Ga (59%), and ^{13}N (33%), the least-squares fit agreed well (goodness-of-fit $R^2 = 0.9825$) with the measured activity. In this calculation, we included ^{13}N because the initial activity reading contained contamination from the activated cellulose [$^{16}\text{O}(p,\alpha)^{13}\text{N}$, $T_{1/2} = 9.97$ min]. Owing to the relatively short half-life ($T_{1/2} = 2.63$ min), ^{64}Ga was not considered in this calculation.

FIG. 6. Time activity curve (measured activity) of gold-coated zinc (Zn@Au) NPs bombarded with 14.15-MeV protons. The curve was least-squares fitted with 3 simple exponential decay curves of ^{66}Ga ($T_{1/2} = 9.50$ h), ^{68}Ga ($T_{1/2} = 67.72$ min), and ^{13}N ($T_{1/2} = 9.97$ min).

MC-simulated proton energies on both top and bottom of the targets are nearly monoenergetic and are in close agreement (within 0.14 MeV) with analytically calculated proton energies (Table I). When NPs were irradiated with 8-MeV incident protons, 173 ± 1 μCi of activity was recorded at 17 min after EOB, but this decreased to 0.5 ± 0.5 μCi over a 5-h period (Table II). These results were compared with the calculated activity assuming the creation of only ^{66}Ga and ^{68}Ga , according to Eq. (1). The notable disagreement in initial activities was suspected mostly due to short-lived isotopes, which were not considered in this calculation; however, the final activities showed better agreement.

Figure 7 shows the gamma ray spectrum of Zn@Au NP samples irradiated with 14.15-MeV protons. After a short delay (2 h after EOB), 511-keV gamma rays from positron emission were dominant [Fig. 7(a)] and most of the other dominant spectral peaks were from decaying ^{66}Ga . The

14.15-MeV proton beam activated gold, and a spectral line from decaying ^{197}Hg appeared. After a longer delay (70 h after EOB), 511-keV gamma rays were no longer dominant, and gamma rays from ^{197}Hg and characteristic x-rays from ^{197}Hg and ^{197}Au became dominant owing to the longer half-life of ^{197}Hg [Fig. 7(b)].

Figure 8 shows the gamma ray spectrum of a Zn@Au NP sample irradiated with 8-MeV protons. The absence of the ^{197}Hg spectral line (134 keV) indicates that 8-MeV protons did not activate gold. The gamma ray spectrum on a logarithmic scale shows that mostly ^{66}Ga and ^{68}Ga were created in this bombardment; however, spectral lines from decaying ^{44}Sc also appear [Fig. 8(a)]. These spectral lines are suspected to be a result of ^{44}Ca impurities in the target bombarded with protons, $^{44}\text{Ca}(p,n)^{44}\text{Sc}$, whose interaction energy threshold is about 4 MeV.

Figure 9 shows the CT, PET, and PET/CT fusion images of the Zn@Au NP sample bombarded with 8-MeV protons. Despite a relatively long delay (12 h) and short PET acquisition time (5 min), a strong PET signal was observed. The unit for PET images is a relative unit, i.e., the quantification of PET images was not possible because neither attenuation correction nor decay correction was applied. Attenuation correction was not applied because CT artifacts from both the Zn@Au NP sample and the aluminum foil may cause distortion in PET images. Decay correction was not applied due to the presence of multiple radionuclides following activation that could not be accounted for using a single decay correction.

Figure 10 shows the MC-calculated secondary electron spectra for NPs bombarded with 160-MeV protons and 250-kVp x-rays. More than 99% of the NP-originated secondary particles were electrons. For both x-rays and protons, the only observed difference between GNPs and Zn@Au NPs was the magnitude of the scored spectrum, which was less than 6%.

Figure 11 shows the photon and electron emission spectra per ^{66}Ga disintegration before and after escaping Zn@Au

TABLE II. Activity measured in gold-coated zinc (Zn@Au) NP irradiated with 14.15 and 8-MeV incident protons.

Variable	First measurement (14.15 MeV)	Second measurement (8.00 MeV)
Initial measured target activity (Specific activity)	168 ± 1 μCi @ 32 min EOB ^a (5.52 mCi/g)	173 ± 1 μCi @ 17 min EOB (7.52 mCi/g)
Initial target activity calculated using Eq. (1)	50 μCi @ 32 min EOB (^{66}Ga : 17%, ^{68}Ga : 83%)	33 μCi @ 17 min EOB (^{66}Ga : 10%, ^{68}Ga : 90%)
Final measured target activity (Specific activity)	2 ± 1 μCi @ 24 h 32 min EOB (0.07 mCi/g)	0.5 ± 0.5 μCi @ 5 h 17 min EOB (0.04 mCi/g)
Final target activity calculated using Eq. (1)	1 μCi @ 24 h 32 min EOB (^{66}Ga : 100%, ^{68}Ga : 0%)	3 μCi @ 5 h 17 min EOB (^{66}Ga : 99%, ^{68}Ga : 1%)
Calculated TTY ^b of ^{66}Ga for 1-h proton bombardment of natural zinc (mCi/ μA)	4.9	0.5
Calculated TTY of ^{66}Ga for 1-h proton bombardment of Zn@Au NPs (mCi/ μA)	0.10	0.01
Calculated activity of Zn@Au NPs bombarded using a 50- μA proton current for 2 h (typical FDG production setting), 24 h after EOB (mCi)	2.37	0.24
Calculated specific activity ^c , 24 h after EOB (mCi/g)	9.84	4.00

^aEOB: end of bombardment.

^bTTY: thick target yield, calculated using Eq. (1).

^cSpecific activity was calculated using target mass calculated in Table III.

Fig. 7. Gamma ray spectrum acquired for 12 h for gold-coated zinc (Zn@Au) NP samples bombarded with 14.15-MeV protons. (a) Gamma ray counting started 2 h after EOB (for $a < 1$ mg sample). A total of 2.05×10^6 counts of 511-keV gamma rays were recorded for the full-width half-maximum of the peak over 511 ± 1.5 keV. (b) Gamma ray counting started 70 h after EOB (for a 28.6-mg sample).

NP. As expected, photons escaping the NP experienced negligible attenuation and energy degradation [Fig. 11(a)]. On the other hand, electrons escaping the NP exhibited moderate self-absorption (17%) and energy degradations [Fig. 11(b)]. Among the escaping electrons, the percentage of primary electrons (from ^{66}Ga disintegration) was 99% and the percentage of secondary electrons (such as photoelectrons and Auger electrons) was 1%.

4. DISCUSSION

In the current study, we developed novel Zn@Au NPs and demonstrated the feasibility of using them as PET-visible NPs. Zn@Au NP samples irradiated with both 14.15 and 8-MeV protons provided excellent 511-keV signals owing to the high interaction cross sections of zinc and the high positron emission branching ratio (B+) of the gallium isotopes resulting from the reaction (Fig. 2). The activated zinc cores in Zn@Au NPs make them ideal for PET imaging for the time scale of minutes, hours, and days owing to distinctly different half-lives of the isotopes resulting from

the reaction: ^{64}Ga , $T_{1/2} = 2.63$ min; ^{68}Ga , $T_{1/2} = 67.72$ min; and ^{66}Ga , $T_{1/2} = 9.50$ h. Biologists studying the *in vivo* targeting of gold and nongold NPs using various time scales can benefit from our findings. The activated gallium isotopes also have relatively low yields for gamma ray (non-511-keV) and electron emissions (Brookhaven National Laboratory National Nuclear Data Center Chart of Nuclides).⁶⁵ This can be beneficial in terms of minimizing the dose due to Zn@Au NPs to the surrounding organ and cellular structures.

Despite the relatively small beam current (5 μA) and irradiation time used in our study, small samples (~ 20 mg) of Zn@Au NPs provided strong enough signals (> 100 μCi of initial activity) for photon counting and clear PET imaging. The expected radioisotope yield was calculated using Eq. (1) and the published cross section data are shown in Fig. 2. To test the accuracy of this calculated radioisotope yield, the thick target yield of ^{66}Ga was calculated for proton bombardments of natural zinc or enriched ^{66}Zn and compared with the published measurement data found from the Brookhaven National Laboratory National Nuclear Data

FIG. 8. Gamma ray spectrum acquired for 1.5 h for a gold-coated zinc (Zn@Au) NP sample (<1 mg) bombarded with 8-MeV protons. Gamma ray counting started 4.5 h after EOB. (a) Logarithmic scale. All solid (green) arrows are gamma ray spectral lines from ^{66}Ga . (b) Linear scale. A total of 680 000 counts of 511-keV gamma rays were recorded for the full-width half-maximum of the peak over 511 ± 1.5 keV.

Center (EXFOR library).⁶⁵ Our calculation agreed with the measured data within $34\% \pm 20\%$ (average $\pm 1\sigma$ standard deviation) (Appendix, Table IV). Thick target yield was calculated using only ^{66}Ga because ^{68}Ga completely decays

out 24 h after EOB, which is possibly the time scale of interest for human applications. The thick target yield of Zn@Au NPs was significantly smaller than that of zinc, obviously owing to the small fraction of zinc in Zn@Au

FIG. 9. Computed tomography (CT; left), positron emission tomography (PET; middle), and PET/CT fusion (right) images of the 23-mg gold-coated zinc (Zn@Au) NP sample irradiated with 8-MeV protons. The PET scan was started 12 h after EOB and acquired for 5 min.

FIG. 10. Monte Carlo-simulated secondary electrons scored at the surface of each NP. (a) NPs were bombarded with 160-MeV protons at the center of modulation. (b) NPs were bombarded with 250-kVp x-rays. GNP: gold nanoparticle. Zn@Au NP: gold-coated zinc nanoparticle.

NPs. However, for the 2-h bombardment with 50- μ A proton current [which is a typical cyclotron setting for ^{18}O and fluorodeoxyglucose (FDG) production], specific activities of 9.84 mCi/g for 14.15-MeV protons and 4.00 mCi/g for 8-MeV protons could be obtained (Table II) although proper target cooling is essential to prevent overheating of the target from high-current proton bombardments for an extended period of time. When higher activities are required, enriched ^{66}Zn can be used as a core material instead of natural zinc to increase the long-term activity (such as 24 h after irradiation) by more than 3-fold. Alternatively, larger-sized zinc cores can be used to increase the activity; however, the possible reduction in the secondary electron production (for radiosensitization) should be taken into consideration.

For consideration for clinical applications, radiation dose from injected Zn@Au NPs should be investigated. For example, Huang *et al.*⁶⁶ showed that a typical PET/CT study using 10 mCi of FDG provided the effective dose from FDG up to 32 mSv which increased the associated lifetime cancer incidence up to 0.5% for the US population. A comparable outcome is expected from injected Zn@Au NPs considering they are almost pure positron

emitters; however, the different physical half-life and biological clearance may give somewhat different outcome. Therefore, a similar internal dose study is warranted to test the radiation safety of Zn@Au NPs for human applications, along with an effort to establish their toxicity profile.

Besides the radiation safety issue, the tumor-NP affinity as well as the biological clearance of NPs will also have to be investigated in future studies. If the tumor affinity and biological clearance of NPs are assumed similar to those of typical PET tracers, achievable specific activities (on the order of 4–10 mCi/g) as calculated in this investigation may be sufficient to image *in vivo* distribution of NPs, considering a clinically relevant scenario of GNP injection on the order of 1 g per patient.²³ PET spatial resolution due to the use of Zn@Au NPs is expected to be similar to that due to the less commonly used PET tracers such as ^{82}Rb , ^{68}Ga , ^{51}Mn , ^{86}Y , and ^{124}I because of relatively high positron energy and range ($E_{\text{mean}} \approx 750$ keV, $R_{\text{mean}} \approx 2$ mm) of ^{68}Ga . On the other hand, Zn@Au NPs would provide PET spatial resolution somewhat inferior to more commonly used ^{18}F ($E_{\text{mean}} \approx 250$ keV, $R_{\text{mean}} \approx 0.6$ mm). Nevertheless, PET spatial resolution achievable with Zn@Au NPs can still be well within

FIG. 11. Energy spectra of the source photons and electrons (with branching ratio $>0.3\%$) of decaying ^{66}Ga (normalized per disintegration which emits 2.2 photons and 0.8 electrons per ^{66}Ga decay) and the changes in the energy spectra after escaping Zn@Au NP. (a) There were negligible attenuation and energy degradation for photons (blue spectral peaks are nearly identical to red spectral peaks in the figure). The percentage of secondary photons was nearly zero. (b) Moderate self-absorption (17%) and energy degradation of primary electrons were noted as shown while escaping Zn@Au NPs. (See color online version.)

the range provided by PET tracers currently available for clinical uses, noting those PET tracers have even greater E_{mean} and R_{mean} .

There were some discrepancies between the measured and calculated target activities, as shown in Table II. Causes for these discrepancies could include cross section uncertainties as well as the result of using the ^{66}Ga dose calibrator setting, although the activated targets contain multiple radioisotopes, including ^{68}Ga and ^{13}N . Additionally, the size variations in the zinc cores were likely to have played a role in these discrepancies. While the calculated target activity was based on the identical zinc core diameter and gold shell thickness, the TEM-measured zinc core and shell thickness had a rather wide range of variation. For example, 1 nm change in gold shell thickness from 4.25–3.25 nm or 5.25 nm could increase or decrease the PET signals by approximately 100%. Also, we suspect that the ^{44}Sc signals arose from the proton activation of calcium (^{44}Ca , natural abundance = 2.1%) that exists as a compound (CaO) of fiberglass in the high-temperature aluminum tape.

The current study is not the first to examine nuclear activation of GNPs or near-GNPs. Neutron activation of GNPs was performed to create radioactive ^{198}Au NPs, whose beta and relatively low energy gamma ray (the mean/median energy of 129/215 keV) emissions were used for therapy while escaping gamma rays were exploited concurrently for *in vivo* imaging using SPECT.^{67,68} Such radioactive GNPs could be beneficial if sufficient NP-tumor affinity was achieved. The Zn@Au NPs that we developed in this study differ from ^{198}Au NPs in that proton-activated Zn@Au NPs are most dominantly positron (511-keV) emitters (even all other less dominant gamma ray peaks are >511 keV) with significantly less beta ray emission (whose electron integral dose is on the order of 100 times smaller) compared with ^{198}Au NPs. Therefore, we expect that proton-activated Zn@Au NPs will deliver a significantly less patient dose or detrimental effects than ^{198}Au NPs when distributed to healthy tissues or organs. In addition, 5 h after EOB, ^{68}Ga in Zn@Au NPs decays to less than 5% of its original activity, whereas ^{66}Ga retained 70% of its original activity. Therefore, to reduce the patient dose even further, Zn@Au NPs can be administered a few hours after EOB because ^{68}Ga contributes only to patient dose, not to PET imaging if imaged after several hours or days.

GNPs have been studied as sensitizers not only for radiotherapy but also for various other treatments, including thermal therapy⁶⁹ and chemotherapy.⁷⁰ Therefore, various GNP-mediated targeting and drug delivery methodologies have become available^{71–75} and some of them are routinely used in laboratories.^{76,77} The Zn@Au NPs that we developed have the same external structure as spherical GNPs and therefore have the potential to be used with the existing GNP-mediated targeting methodologies. Owing to the nature of NPs, which passes through many cell and organ membranes, including the blood–brain barrier, GNPs are also sought for brain imaging and the treatment of brain tumors.^{8,78,79} Zn@Au NPs may serve the same purpose in the treatment

of brain tumors, with the added benefit of PET-mediated neuromolecular imaging.

There are several limitations to the current study. For instance, there was no verification of the quality of the gold coating or its stability other than TEM images. Additionally, post-irradiation TEM images were not taken to test the integrity of Zn@Au NPs after proton bombardment. However, Pérez-Campaña *et al.*⁴⁹ showed that proton bombarded TiO_2 NPs were stable and the changes in the size distribution of proton-bombarded TiO_2 NPs were minimal (i.e., before/after bombardment $7.8 \pm 2.6/7.4 \pm 2.9$ nm) for similar measurement conditions (target current@5 μA , irradiation time@6 min). Additionally, although photon and electron emissions from radioactive Zn@Au NPs were characterized by MC simulations in this study, effective dose (or risk) due to the presence of radioactive Zn@Au NPs in human body and organs were not addressed either analytically or using MC simulation. Besides, for GNP-mediated radiosensitization, the difference or similarity between solid GNPs and radioactive Zn@Au NPs of the same size (i.e., the same outer diameter) cannot be fully elucidated, solely based on the comparison of their secondary electron spectra, because of some unknown biological/physical effects *in vitro* and *in vivo* due to the radioactivity of Zn@Au NPs. Future studies will be necessary to address the above and other issues noted from the current study.

5. CONCLUSIONS

We successfully synthesized PET-imageable Zn@Au NPs. To the best of our knowledge, this is the first such attempt to show the feasibility of bimetallic hybrid GNPs with two distinct properties that can be taken advantage of. PET-visible Zn@Au NPs may be useful to study GNP tumor-targeting in both *in vivo* and *in vitro* settings. They also have the potential to be used for GNP-mediated radiosensitization (or GNP-aided radiotherapy) guided by PET imaging. If future research efforts are successful, we envision that these hybrid GNPs may improve not only radiotherapy but also molecular imaging of various human anatomical sites, including the brain.

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CONFLICT OF INTEREST DISCLOSURE

The authors have no COI to report.

APPENDIX: TARGET MASS CALCULATION AND THE VERIFICATION OF THICK TARGET YIELD CALCULATION WITH PUBLISHED EXPERIMENTAL DATA

TABLE III. Gold-coated zinc (Zn@Au) NP mass calculation for a cylindrical target with a 0.8-cm diameter.

Variable	First measurement (14.15 MeV)	Second measurement (8.00 MeV)
Target entrance energy (MeV)	14.15	8.00
Target exit energy [threshold energy of $^{66}\text{Zn}(p, n)^{66}\text{Ga}$ interaction] (MeV)	5.00	5.00
Thick target thickness (TTT^a) (g/cm ²)	0.476 86	0.1226
Target mass ($TTT \times \pi \times 0.4^2$) (g)	0.239 7	0.0616

^a TTT was calculated as the projected ranges of protons between the entrance and exit energies in a thick target of Zn@Au NPs.

TABLE IV. Calculation of ^{66}Ga thick target yield for proton bombardments of natural zinc or enriched ^{66}Zn [using Eq. (1)] and comparison with published data.^a

Proton energy (MeV)	Target	Calculation	Published data	References
10.4	99.99% ^{66}Zn	1.80 mCi/($\mu\text{A} \cdot \text{h}$) ^b	1.01 mCi/($\mu\text{A} \cdot \text{h}$)	Isshiki <i>et al.</i> , 1984
11.0	100% ^{66}Zn	114.05 mCi/ μA	105 mCi/ μA	Nickles, 1991
13.5	100% ^{66}Zn	15.54 mCi/($\mu\text{A} \cdot \text{h}$)	10.703 mCi/($\mu\text{A} \cdot \text{h}$)	Tarkanyi <i>et al.</i> , 1984
16.0	28% ^{66}Zn	6.23 mCi/($\mu\text{A} \cdot \text{h}$)	2.808 mCi/($\mu\text{A} \cdot \text{h}$)	Abe <i>et al.</i> , 1984

^aPublished data are from the Brookhaven National Laboratory Experimental Nuclear Reaction Database. Full author and journal information can be found in the EXFOR database.

^bmCi/ μA : thick target saturation yield; mCi/($\mu\text{A} \cdot \text{h}$): thick target yield per hour.

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