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Radioluminescent Gold Nanocages with Controlled Radioactivity for Real-time *In Vivo* Imaging

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Abstract

Cerenkov luminescence imaging based on light emission from the decay of radionuclides has recently drawn great interest in molecular imaging. In this paper, we report, for the first time, the Cerenkov luminescence phenomenon of ¹⁹⁸Au isotope, as well as a facile route to the preparation of radioluminescent Au nanocages without additional radiolabeling or dye conjugation. The specific radioactivity of the Au nanocages could be easily and precisely controlled by varying the concentration of H¹⁹⁸AuCl₄ precursor used for the galvanic replacement reaction. The direct incorporation of ¹⁹⁸Au atoms into the structure of Au nanocages enabled the ability of accurate analysis and real-time imaging *in vivo*. Furthermore, under biological conditions, the radioactive Au nanocages were shown to emit light with wavelengths in the visible and near-infrared regions, enabling luminescence imaging of the whole mice *in vivo*, as well as the organs *ex vivo*. When combined with their favorable scattering and absorption properties in the near-infrared region, the radioactive Au nanocages can serve as a new platform for multimodality imaging and will have a significant impact on both small animal and clinical imaging.

Keywords

Gold nanocages; cancer imaging; Cerenkov luminescence imaging; radioactive

There is a strong need to develop contrast agents for optical imaging and related applications in medical diagnostics.^{1–3} Among all contrast agents studied thus far, luminescent nanostructures have attracted particular interest thanks to their unique advantages in terms of tunable optical spectra, high sensitivity, and superb photostability against photobleaching.^{4, 5} In addition, luminescent nanostructures can be synthesized in a controllable manner with desired sizes, shapes, and surface properties, making them better suited for biological applications *in vivo*.^{6–8} To date, a large number of different nanostructures such as

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ASSOCIATED CONTENT

Supporting Information

Additional information and figures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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semiconductor nanocrystals, graphene, nanodiamond, metal nanostructures, and rare-earth nanoparticles have all been synthesized and evaluated as contrast agents for luminescence imaging.^{9–12}

Cerenkov luminescence is referred to as the light emitted during the decay of a radionuclide.^{13–15} It has received great interest as an emerging modality for molecular imaging owing to its ability to bridge nuclear imaging (*e.g.*, positron emission tomography or PET and single-photon emission computed tomography or SPECT) with optical imaging, which is much less expensive, easier to operate, and of higher throughput than its nuclear counterparts.¹⁶ In addition, compared to conventional luminescence, Cerenkov luminescence is not limited by the penetration depth of excitation light since no excitation light will be involved. As a result, the auto fluorescence background from the normal tissues can be minimized, which can further increase the signal-to-noise ratio and reduce the dose of radionuclide for *in vivo* imaging. Over the past few years, many α - or β -particle emitting radionuclides, such as ¹⁸F, ¹³¹I, ⁶⁴Cu, ^{99m}Tc, ⁹⁰Y, ¹¹¹In, ¹⁷⁷Lu, and ²²⁵Ac, have been shown to emit low-energy light in the visible and near-infrared regions, which have been exploited for Cerenkov radioluminescence imaging.^{16–19} When combined with nanostructured materials, this imaging platform is expected to play an important role in future diagnostic applications. Normally, the radioactive isotope is attached to the surface of nanostructures through a ligand or direct encapsulation.^{20, 21} This approach is, however, troubled by various problems, including change of surface properties for the nanostructures, requirement of complex procedures for synthesis and purification, and possible instability of the conjugates in biological system and therefore false diagnostic information owing to the protein transchelation and dehalogenation process.^{22–24} Alternatively, the radioactive isotope could be incorporated into the structure of the nanomaterials through neutrons (or other high energy particles) bombardment.²⁵ However, this approach is often plagued by the lack of precise control over radioactivity generated during the production, and possible alternation of physicochemical properties to the nanostructures such as aggregation caused by the high energy radiation.²⁵

In this work, we demonstrated, for the first time, the Cerenkov luminescence phenomenon for the ¹⁹⁸Au isotope ($\beta_{\max} = 0.96$ MeV, $t_{1/2} = 2.7$ days), which has been widely employed as therapeutic isotope for radiation therapy owing to the high energy.²⁶ We then developed a new approach to synthesize ¹⁹⁸Au-doped Au nanostructures for Cerenkov luminescence imaging, with other physicochemical properties essentially identical to those of the conventional non-radioactive Au nanostructures. We chose Au nanocages (AuNCs) as a typical example for the proof-of-concept experiments because they have been widely used as theranostic agents for a variety of biomedical applications owing to their attractive features such as biocompatibility, easy surface modification, tunable optical scattering/absorption peaks in the near-infrared region for photothermal effect, and availability of a broad range of sizes.²⁷ Therefore, the radioactive AuNCs can serve as multifunctional theranostic probe for a variety of applications. The synthesis strategy is similar to what was used in recent studies reported by one of us (C. Cutler) and Zheng, where ions of ¹⁹⁸Au were used as a precursor to the formation of radioactive Au nanoparticles.^{28,29} This simple and straightforward method allowed us to easily control the radioactivity of the final products. Moreover, the β^- energy of ¹⁹⁸Au is significantly higher than 0.26 MeV, which is the threshold of kinetic energy required for Cerenkov radiation in biological tissue, ensuring high throughput emission of Cerenkov luminescence.¹⁶

We started the synthesis by irradiating a piece of non-radioactive ¹⁹⁷Au foil with neutrons to generate radioactive ¹⁹⁸Au. The foil was then dissolved in aqua regia to obtain a mixture of H¹⁹⁸AuCl₄ and HAuCl₄. The as-prepared mixture was highly acidic, together with byproducts such as NO₂ and NO, so it could not be directly used for the synthesis of Au

nanostructures.^{26, 27} This mixture was thus purified by repeated heating at 130 °C to get rid of the acids and byproducts.³⁰ The final product could be used as a precursor for preparing ¹⁹⁸Au-doped AuNCs with controlled radioactivity *via* the galvanic replacement reaction with Ag nanocubes (see the Supporting Information for details).³¹ The localized surface plasmon resonance (LSPR) peak of the AuNCs was tuned to 760 nm (Figure 1a). Figure 1b shows the TEM image of a typical product, which was taken after the ¹⁹⁸Au had completely decayed. The AuNCs had an average edge length of 33 nm. The TEM image confirmed that the presence of ¹⁹⁸Au in the crystal lattice did not cause any changes to the shape and/or structure of the resultant AuNCs in comparison with the samples prepared using HAuCl₄ alone.³²

The radiochemical purity of the ¹⁹⁸Au-doped AuNCs was confirmed by radioactive thin layer chromatography (Figure 1c) and fast protein liquid chromatography (Figure S1). It is shown that all the radioactivity of the AuNCs was associated with the ¹⁹⁸Au atoms in their walls without any contribution from unbound ¹⁹⁸Au³⁺, indicating the structural integrity of the ¹⁹⁸Au-doped AuNCs. Further, mouse serum stability of ¹⁹⁸Au-doped AuNCs showed no dissociated ¹⁹⁸Au³⁺ over a week, indicating the stability of integrated ¹⁹⁸Au in the nanostructure. We could easily control the specific radioactivity of the ¹⁹⁸Au-doped AuNCs by varying the initial amount of H¹⁹⁸AuCl₄ (or the radioactivity concentration) added into the reaction system. As shown in Figure 1d, the specific radioactivity of the resultant ¹⁹⁸Au-doped AuNCs was linearly increased from 17 to 3,700 μCi/nmol as the initial radioactivity concentration of H¹⁹⁸AuCl₄ was increased from 0.32 to 70 μCi/mL.

Next we investigated the ability to visualize and quantify the Cerenkov luminescence emitted from the ¹⁹⁸Au-doped AuNCs using an *in vitro* phantom study. Aqueous suspensions of the ¹⁹⁸Au-doped AuNCs at various radioactivities (1.56 – 50 μCi, with AuNCs concentrations in the range of 1.3 – 41 pmol) were placed in a 96-well black plate and imaged using a conventional small-animal IVIS Lumina II XR optical imaging system. Clearly, even with radioactivity as low as 1.56 μCi, a good (>10) signal-to-noise ratio was observed with acquisition time of 5 min (Figure 2a), indicating high Cerenkov luminescence sensitivity of the ¹⁹⁸Au-doped AuNCs. Furthermore, a plot of the total flux extracted from the phantom image *vs.* the amount of activity showed a linear relationship in the range of activity tested, revealing the potential of Cerenkov luminescence imaging based on the ¹⁹⁸Au-doped AuNCs for quantitative imaging analysis (Figure 2b). Similar to other radionuclides, the ¹⁹⁸Au displayed a continuous luminescent spectrum with a broad maximum at 500 – 550 nm and monotonic decline out to 800 nm (Figure 2c), which can be attributed to the inverse dependence of Cerenkov radiation intensity on wavelength.

We next explored the *in vivo* biodistribution of the ¹⁹⁸Au-doped AuNCs in mice bearing EMT-6 tumors. This EMT-6 breast cancer model is known for rapid growth together with tumor angiogenesis and high vessel permeability, allowing for accumulation of nanoparticles in the tumor through the enhanced permeability and retention (EPR) effect.^{33, 34} The ¹⁹⁸Au-doped AuNCs were modified with poly(ethylene glycol) (PEG, MW ≈ 5,000) for its ability to improve the half-life in the bloodstream.³⁵ In a typical study, 100 μL of the PEGylated AuNCs in saline (1.7 × 10¹² particle/mice or 3.5 μCi/mice) was intravenously administered to a mouse through tail vein injection. The organs of interest were collected at 1, 6 and 24 h post injection for gamma counting. Analyses of ¹⁹⁸Au radioactivity revealed that the PEGylated AuNCs exhibited slow blood clearance with 36.2 ± 6.4 %ID/g (the percentage of the injected dose per gram of tissue) at 1 h, and 22.1 ± 4.0 %ID/g at 6 h post injection, respectively (Figure 3a). Moreover, there was a significant accumulation in the reticuloendothelial system (RES) including liver and spleens, while the other organs exhibited very low uptake. Over 10.0 %ID/g of the PEGylated AuNCs was retained in the tumor at 6 h post injection; the uptake reached 15.3 ± 2.9%ID/g at 24 h post

injection, which was significantly higher than previously reported data, indicating the great potential for cancer photothermal treatment.³⁶ Furthermore, the tumor-to-blood ratio (Figure 3b) and tumor-to-muscle ratio (Figure 3c) monotonically increased from 0.12 ± 0.02 and 3.99 ± 2.56 at 1 h to 3.81 ± 1.08 and 26.5 ± 12.0 at 24 h, respectively. These pharmacokinetic features confirmed the excellent accumulation of the ^{198}Au -doped AuNCs in tumors after PEGylation.

We also evaluated the feasibility of using Cerenkov luminescence with ^{198}Au -doped AuNCs for *in vivo* imaging in mice bearing EMT-6 tumors. In this case, the PEGylated AuNCs were intravenously injected into mice bearing EMT-6 tumor xenografts in the right hind legs ($60 - 70 \mu\text{Ci}$ per mouse) and bioluminescence images were sequentially captured using the IVIS living imaging system. Noticeably, the PEGylated AuNCs rapidly accumulated in the tumor at 0.25 h post injection (Figure 4). Significant accumulations in the spleen and liver were also observed, in consistence with the results obtained in biodistribution study (Figure 3a). As confirmed by semi-quantitative analysis of the region-of-interest (ROI) after decay correction, the bioluminescence intensities at the tumor site increased constantly up to 24 h, indicating the potential use of this new imaging platform for quantitative analysis (Figure S2). To further characterize the distribution of the PEGylated AuNCs, the same mice were sacrificed at 48 h post injection, and *ex vivo* bioluminescence images were recorded from the tumor as well as major organs. The Cerenkov luminescence imaging signals from the *ex vivo* organ-of-interest correlated well with the results obtained in biodistribution study, showing high RES system uptakes, low kidney clearance, and considerable tumor accumulations (Figure S3). It is worth mentioning that no adverse reaction was observed at the administered doses during all experiments. Additionally, the dosimetry calculated from biodistribution studies showed low radiation burden on individual organs (Table S1).

In summary, we have demonstrated, for the first time, a facile synthesis of radioluminescent, ^{198}Au -doped nanocages and examined their potential use as Cerenkov luminescence imaging agents. The specific radioactivity of the nanocages could be controlled by varying the initial concentration of the $\text{H}^{198}\text{AuCl}_4$ precursor. The direct incorporation of ^{198}Au into the walls of nanocages ensured good stability for imaging *in vivo* and accurate analysis. The AuNCs were shown to emit luminescence with continuous wavelengths in the visible and near-infrared regions, enabling luminescence imaging of the whole mice *in vivo*, as well as their organs *ex vivo* for direct gamma counting. The synthetic approach can also be readily extended to prepare radioluminescent Au nanostructures with a variety of different shapes or structures. More importantly, the ^{198}Au nanocages showed high accumulation in tumor for cancer treatment. The nuclear imaging capability of ^{198}Au can be combined with the unique scattering/absorption properties of Au nanostructures to develop theranostic nanoprobe for oncological applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

a) Normalized UV-vis spectra taken from aqueous suspensions of the Ag nanocubes and ^{198}Au -doped AuNCs (after the isotope had decayed for one month), respectively. b) TEM image of the ^{198}Au -doped AuNCs with an average edge length of 33 nm. The image was recorded after the ^{198}Au had decayed for one month. c) Paper chromatography analyses of the $\text{H}^{198}\text{AuCl}_4$ and ^{198}Au -doped AuNCs immediately after preparation. d) Plot of specific activity of the AuNCs as a function of the specific activity of $\text{H}^{198}\text{AuCl}_4$ solution employed for the galvanic replacement reaction.

Figure 2.

a) Optical images of aqueous suspensions of ^{198}Au -doped AuNCs with different levels of radioactivity in a 96-well plate. The images were taken using the IVIS Lumina II XR system from Caliper. b) Plot of photon flux versus radioactivity for aqueous suspensions of the ^{198}Au -doped AuNCs in a 96-well plate. c) Luminescence intensities at different wavelengths recorded from an aqueous suspension of the ^{198}Au -doped AuNCs at a radioactivity of 200 $\mu\text{Ci/mL}$.

Figure 3.

a) Biodistribution of the ^{198}Au -doped AuNCs in mice bearing EMT-6 tumors following tail vein injection. b) Tumor to blood ratios of the ^{198}Au -doped AuNCs in mice at different times post injection. (c) Tumor to muscle ratios of the ^{198}Au -doped AuNCs in mice at different times post injection. The AuNCs were 33 nm in edge length and covered by poly(ethylene glycol) (PEG) chains of 5,000 in molecular weight.

Figure 4.

Bioluminescence imaging with the IVIS spectrum system from Caliper. Representative luminescent images (with excitation filter closed and emission filter open) of a mouse bearing EMT-6 tumors after tail vein injection of the PEGylated ^{198}Au -doped AuNCs (64 μCi per mouse). The images were taken at 0, 0.25, 2, 6, 24, and 48 h post injection, respectively.