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Renal-Clearable Ultra-Small Coordination Polymer Nanodots for Chelator-Free ^{64}Cu -Labeling and Imaging-Guided Enhanced Radiotherapy of Cancer

Sida Shen¹, Dawei Jiang^{2,3}, Liang Cheng^{1,*}, Yu Chao¹, Kaiqi Nie¹, Ziliang Dong¹, Christopher J. Kutyreff², Jonathan W. Engle², Peng Huang³, Weibo Cai^{2,*}, and Zhuang Liu^{1,*}

¹Institute of Functional Nano & Soft Materials (FUNSOM), Collaborative Innovation Center of Suzhou Nano Science and Technology, Soochow University, Suzhou, Jiangsu 215123, China

²Departments of Radiology and Medical Physics, University of Wisconsin-Madison, Wisconsin 53705, United States

³Guangdong Key Laboratory for Biomedical Measurements and Ultrasound Imaging, School of Biomedical Engineering, Health Science Center, Shenzhen University, Shenzhen 518060, China

Abstract

Developing tumor-homing nanoparticles with integrated diagnostic and therapeutic functions, and in the meanwhile could be rapidly excreted from the body, would be of great interests to realize imaging-guided precision treatment of cancer. In this study, a kind of ultrasmall coordination polymer nanodots (CPNs) based on the coordination between tungsten ions (W^{VI}) and gallic acid (W-GA) was developed *via* a simple method. After polyethylene glycol (PEG) modification, PEGylated W-GA (W-GA-PEG) CPNs with an ultrasmall hydrodynamic diameter of 5 nm were rather stable in various physiological solutions. Without the need of chelator molecules, W-GA-PEG CPNs could be efficiently labeled with radioisotope $^{64}\text{Cu}^{2+}$, enabling positron emission tomography (PET) imaging, which reveals efficient tumor accumulation and rapid renal clearance of W-GA-PEG CPNs upon intravenous injection. Utilizing the radio-sensitizing function of tungsten with strong X-ray absorption, such W-GA-PEG CPNs were able to greatly enhance the efficacy of cancer radiotherapy in inhibiting the tumor growth. With fast clearance and little long-term body retention, those W-GA-PEG CPNs exhibited no appreciable *in vivo* toxicity. This study presents a type of CPNs with excellent imaging and therapeutic abilities as well as rapid renal clearance behavior, promising for further clinic translation.

Graphical abstract

*Corresponding Authors. lcheng2@suda.edu.cn (L. Cheng), zliu@suda.edu.cn (Z. Liu), wcai@uwhealth.org (W.B. Cai).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Materials characterization and supplementary figures (PDF).

The authors declare no competing financial interest.

Keywords

coordination polymer nanodots; chelator-free ^{64}Cu -labeling; positron emission tomography (PET) imaging; radiotherapy; rapid renal clearance

Cancer nanomedicine by using various types of nanomaterials to realize tumor-specific imaging and therapy has received tremendous attention in the past few decades, showing many encouraging results in both preclinical research and clinical applications.^{1–9} For instance, many types of nanoparticles with excellent optical, magnetic or ultrasonic properties have been extensively investigated as imaging probes for *in vivo* tumor detection.^{10,11} To reduce side effects and improve the efficacy of cancer treatment, numerous types of nanoscale drug delivery systems have been explored for tumor-targeted delivery of various therapeutic agents including chemotherapeutics for chemotherapy, therapeutic nuclear acids for gene therapy, as well as photosensitizers for photodynamic therapy.^{12–14} As far as cancer radiation therapy (RT) is concerned,¹⁵ a number of nanoagents containing high-Z elements such as bismuth,¹⁶ gold^{17,18} and rare earth elements,¹⁹ which are able to absorb high energy ionization radiation (*e.g.* X-ray) to act as radiosensitizers, have also been developed in recent years to enhance the efficacy of cancer radiotherapy. However, after systemic administration, a large amount of nanomaterials would be trapped in the reticuloendothelial system organs such as liver and spleen, resulting in the inefficient excretion of those nanoparticles from the body. Therefore, the long-term body retention of nanoparticles, particularly those inorganic and non-biodegradable ones, would lead to concerns regarding their potential long-term toxicity, impeding clinical translations of many nanomaterials.^{20,21}

To avoid long-term body retention of nanomaterials, ultrasmall nanostructures (smaller than 6–8 nm) that could pass through the kidney filtration and then be excreted *via* renal clearance have attracted a great deal of interests in the area of nanomedicine in recent years.^{22–26} Notably, for many ultrasmall nanostructures, although their core sizes are smaller than 6 nm, their actual hydrodynamic sizes within the plasma could be much larger owing to the formation of protein corona on top of those nanoparticles or even their self-aggregation, hampering their efficient renal clearance.^{27,28} On the other hand, integration of multiple imaging and therapy functions within such ultrasmall nanoscale structures often would require relatively complicated design and fabrication processes.^{29,30} Hence, it would be of great interests to develop simple methods for the fabrication of multifunctional nanoparticles with ultrasmall hydrodynamic sizes in physiological conditions such as the plasma

environment, so as to enable rapid renal clearance of those nanoparticles from the body, after their missions in cancer imaging and enhanced therapy are accomplished.

Nanoscale coordination polymers consisting of metal ions or clusters linked by organic ligands *via* the self-assembly process,^{31,32} have shown many excellent properties, such as biodegradability, ease of surface functionalization, well-defined morphology, and compositional diversity.^{33–35} Herein, we report a kind of ultrasmall W^{VI}-gallic acid (W-GA) coordination polymer nanodots (CPNs) with uniform morphology by a facile solution method. After surface modification by polyethylene glycol (PEG), the obtained PEGylated W-GA (W-GA-PEG) CPNs became rather stable in various physiological solutions with the hydrodynamic diameter at about ~5.6 nm, satisfying the requirement for renal filtration. Intriguingly, such W-GA-PEG CPNs could be labeled with radioisotope ⁶⁴Cu *via* a chelator-free method with high labeling efficiency and stability. The obtained ⁶⁴Cu-W-GA-PEG CPNs could be used for *in vivo* positron emission tomography (PET) imaging, which uncovered the efficient retention of those CPNs in the tumor as well as their rapid renal excretion after intravenous (*i.v.*) injection. Owing to the existing of W within such small CPNs to absorb X-ray, such W-GA-PEG CPNs could act as an effective radio-sensitizer that can remarkably enhance the efficacy of radiation-induced cancer cell killing. As the results, it was found in our mouse tumor model experiments that the efficacy of *in vivo* radiotherapy could be greatly improved with the help of W-GA-PEG CPNs, which after their therapeutic function was accomplished could be rapidly eliminated from the body *via* renal clearance without any obvious long-term toxicity. Thus, our work presents a kind of CPNs with ultrasmall sizes to allow rapid body clearance, as well as effective imaging and cancer therapy enhancement functions, promising for further translational research.

Result and Discussion

The synthesis and surface modification of W-GA CPNs was illustrated in Figure 1a.³⁶ In brief, tungsten hexachloride (WCl₆) was firstly dissolved in water and mixed with polyvinyl pyrrolidone (PVP, 10 kDa). After one hour of incubation, gallic acid (GA) was slowly introduced into the above solution, whose color quickly became brown, indicating the successful formation of CPNs *via* the interaction between W^{VI} and GA. The as-synthesized W-GA product, which could pass through the membrane of 100 kDa molecular weight cut-off (MWCO) filters (Millipore) (Figure 1b, **insert**), showed uniform sizes of 1.61 ± 0.23 nm as observed under transmission electron microscope (TEM). No well-shaped nanodots were formed in our control synthesis experiments without the addition of either PVP or GA, indicating the critical interaction between W^{VI} and GA, as well as the important role of PVP to control the sizes of the obtained nanostructure (Supporting Figure S1&2). Furthermore, atomic force microscopy (AFM) was also carried out to characterize the size and morphology of the obtained W-GA CPNs, whose diameter was determined to be around ~1.55 nm, in good agreement with the TEM results (Figure 1c).

We then carefully characterized the obtained W-GA CPNs. Such W-GA CPNs showed an amorphous structure as analyzed by the X-ray diffractometer (XRD) (Supporting Figure S3). The energy-dispersive X-ray spectroscopy and X-ray photoelectron spectroscopy (XPS) data both evidenced the existence of tungsten in W-GA CPNs (Supporting Figure S4&5).

Moreover, the two strong binding energy peaks at 35.82 eV and 38.05 eV, which could be attributed to W 4f_{7/2} and W 4f_{5/2} of W⁶⁺, respectively, were found in the spectrum of W-GA CPNs (Figure 1d). In addition, as illustrated by the X-ray absorption fine structure analysis (XANES) (Figure 1e), the spectra of W in W-GA CPNs matched well with that in WO₃, consistent to the XPS characterization data. Furthermore, by calculating the peak area of W-GA and comparing it with that of WO₃ (Supporting Figure S6), we could estimate that each W atom may be coordinated with six oxygen atoms within W-GA CPNs.³⁷ The infrared band of GA at 1,250 cm⁻¹, which corresponded to the OH-C stretching band in the Fourier transform infrared (FTIR) spectrum (Figure 1f), decreased significantly in the FTIR spectrum of W-GA CPNs, suggesting the coordination between HO-C of GA and W^{VI}.

In order to apply the synthesized CPNs for biomedical applications, the obtained W-GA CPNs were conjugated with amine-terminated polyethylene glycol (NH₂-PEG, MW=5 K) to avoid self-aggregation and serum protein adsorption for CPNs in various physiological solutions. After surface modification, the structure of W-GA CPNs didn't change much according to Supporting Figure S7, while the hydrodynamic size of W-GA CPNs measured by dynamic light scattering (DLS) was increased to ~5.6 nm, which appeared to be larger than that measured by TEM and AFM and could be attributed to the outer polymer coating layer on the nanodots' surface. However, even with PEGylation, the hydrodynamic size of W-GA-PEG CPNs was still below the renal filtration threshold (DLS <10 nm) (Figure 1g). The FTIR spectra and thermogravimetric analysis (TGA) of W-GA-PEG CPNs further confirmed the successful conjugation of PEG with the mass percentage of 27.35% (Supporting Figure S8). After incubation with different physiological solutions including H₂O, phosphate buffered saline (PBS), and serum for a week, W-GA-PEG CPNs still exhibited excellent stability (Supporting Figure S9&10), while W-GA CPNs without surface PEG modification aggregated after 24 h in PBS solution (Supporting Figure S11).

As tungsten is a high-Z element with strong X-ray absorption, several W-containing nanostructures have previously been demonstrated to be effective radio-sensitizers to enhance cancer cell damages triggered by ionizing radiation. We thus would like to evaluate the radio-sensitizing effect of W-GA-PEG CPNs in this study. Firstly, four different cell lines including murine breast cancer 4T1 cells, murine colon cancer CT26 cells, human epithelial carcinoma HeLa cells, and mouse embryonic fibroblast NIH3T3 cells were cultured with various concentrations of W-GA-PEG CPNs for 24 h. No obvious cytotoxicity induced by W-GA-PEG CPNs was observed even at high concentrations of W-GA-PEG up to 0.2 mg/mL as determined by the standard methylthiazolyl tetrazolium (MTT) assay (Figure 2a). Afterwards, the clonogenic survival assay was carried out to evaluate the *in vitro* radio-sensitizing effect of W-GA-PEG CPNs. Compared to cells in the absences of nanodots, 4T1 cancer cells cultured with W-GA-PEG CPNs showed significantly reduced clone numbers under the same doses of X-ray radiation (Figure 2b). Moreover, as revealed by immunofluorescently staining of γ -H₂AX, a double-strand DNA breaks marker,³⁸ addition of W-GA-PEG could indeed result in greatly increased DNA damages under X-ray radiation at a dose of 6 Gy (Figure 2c). Therefore, our results clearly evidenced that W-GA-PEG CPNs could act as a highly effective radio-sensitizer to improve the efficacy of radiation therapy, probably attributing to the ability of W to absorb X-ray and generate secondary and Auger electrons to damage cells.¹⁹

Among various imaging modalities, positron emission tomography (PET) imaging with excellent imaging sensitivity and superior tissue penetration has been widely used in the clinic for disease diagnosis and treatment monitoring.^{39,40} However, to label biomolecules or nanoparticles with radioisotopes ions such as ⁸⁹Zr and ⁶⁴Cu to enable PET imaging, they are usually conjugated with chelator molecules like 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), or desferrioxamine (DFO),^{41–43} which may affect the surface chemistry of the biomolecules or nanoparticles to be labeled. Thus, chelator-free radiolabeling techniques as an alternative method to label nanoparticles have become rather attractive recently.⁴⁴ Motivated by the high affinity between metal ions and the phenolic hydroxyl groups of GA,³⁶ we thus hypothesized that W-GA-PEG CPNs might be labeled with ⁶⁴Cu *via* the chelator-free method upon simple mixing. In our experiment, ⁶⁴CuCl₂ was mixed with W-GA-PEG CPNs at 37 °C under constant stirring for an hour. After removing excess free ⁶⁴Cu by ultrafiltration, as much as 88.34% ± 4.33% of added ⁶⁴Cu was successfully labeled onto W-GA-PEG CPNs (Figure 3b), possibly due to the anchoring of Cu²⁺ with phenol groups of GA (Figure 3a). The serum stability test of ⁶⁴Cu-W-GA-PEG was carried out by incubating ⁶⁴Cu-W-GA-PEG CPNs with complete serum at 37 °C for 24 h, little detachment of ⁶⁴Cu was observed, indicating the excellent labeling stability of ⁶⁴Cu-W-GA-PEG CPNs by such a simple chelator-free method (Figure 3c).

Next, *in vivo* PET imaging was conducted to track ⁶⁴Cu labeled W-GA-PEG CPNs after intravenous (*i.v.*) injection into 4T1-tumor-bearing Balb/c mice (10 MBq per mouse) by the micro PET Inveon rodent model scanner. Obvious tumor contrast was observed after injection of ⁶⁴Cu-W-GA-PEG CPNs (Figure 3d). Based on time-dependent PET imaging data, the tumor uptake of ⁶⁴Cu-W-GA-PEG reached the highest level of 5.8 ± 0.50 percent-of-injected-dose-per-gram-tissue (%ID/g) at 4 h post injection (*p.i.*), likely due to the enhanced permeability and retention effect (EPR) of solid tumors (Figure 3d&e). In addition, the levels of ⁶⁴Cu-W-GA-PEG retained in the liver showed significant decrease over time, while that in kidneys still remained at high levels after 24 h, indicating the gradual clearance of ⁶⁴Cu-W-GA-PEG CPNs most likely *via* the renal pathway (Figure 3e, Supporting Figure S12).

To confirm the *in vivo* behaviors of W-GA-PEG CPNs and the PET imaging data based on ⁶⁴Cu labeling, time-dependent blood circulation and biodistribution studies were carried out on 4T1-tumor-bearing mice after *i.v.* injection of W-GA-PEG CPNs by using inductively coupled plasma atomic-emission spectroscopy (ICP-AES) to measure the levels of W in different organs (Figure 3f). As shown in Supporting Figure S13, the blood circulation half-lives of W-GA-PEG were determined to be 0.29 ± 0.14 h and 3.62 ± 0.47 h, for the first ($t_{1/2}(\alpha)$) and the second ($t_{1/2}(\beta)$) phases, respectively, as fitted by a two-compartment model. The tumor accumulation of W-GA-PEG CPNs reached the peak level of 4.63 ± 0.58 % ID/g at 4 h, in reasonable agreement with the PET imaging data with ⁶⁴Cu-W-GA-PEG. Moreover, despite retention of W in reticuloendothelial system (RES) such as liver and spleen, the W levels in those organs showed time-dependent decrease over time, suggesting the efficient clearance of W-GA-PEG CPNs. Notably, the biodistribution data based on ⁶⁴Cu radioactivities (Supporting Figure S14) matched notably well with that determined by ICP-AES (Figure 3f), indicating the great radiolabeling stability of ⁶⁴Cu-W-GA-PEG *in vivo*.

Next, we would like to employ W-GA-PEG CPNs for *in vivo* radiation therapy. Mice bearing 4T1 tumors (volume $\sim 70 \text{ mm}^3$) were randomly divided into four groups (n= 5 per group): Group 1: control; Group 2: X-ray alone; Group 3: W-GA-PEG; Group 4: W-GA-PEG + X-ray. The dose of W-GA-PEG CPNs in group 3 and 4 was 20 mg/kg, while that of X-ray was 6 Gy. Considering that the tumor uptake of such CPNs would reach the peak level at 4 h *p.i.* as revealed by *in vivo* PET imaging, X-ray radiation in group 4 was conducted 4 h post *i.v.* injection of W-GA-PEG CPNs. After various treatments, the tumor sizes in each group were measured by a digital caliper post treatment (Figure 4a, Supporting Figure S15). As expected, W-GA-PEG CPNs alone exhibited no significant effect on tumor growth, while tumors in group 2 (X-ray alone) showed partly inhibited growth. The most remarkable inhibition on tumor growth was observed in group 4 (W-GA-PEG + X-ray), in which mice were *i.v.* injected with W-GA-PEG CPNs and then irradiated by X-ray. Moreover, the mice in the treated groups (W-GA CPNs + RT) showed significantly prolonged survival time compared to the other three control groups including RT alone (Figure 4b). Our results clearly demonstrated the excellent *in vivo* radiotherapy enhancement effect of such W-GA-PEG CPNs. In order to further investigate the antitumor efficacies of different groups, tumors were harvested 2 days after receiving various treatments and stained by hematoxylin and eosin (H&E) (Figure 4c). Severe damages of tumor cells could be easily observed after W-GA-PEG CPNs enhanced radiotherapy, while tumor cells with radiotherapy alone were partly damaged. In addition, no obvious side effects on mice were noticed after the W-GA-PEG CPNs enhanced radiotherapy, as revealed by the body weight and the histology analysis of major organs collected 20 days after treatments (Supporting Figure S16&17). Therefore, W-GA-PEG CPNs with efficient tumor retention peaked at 4 h post injection, as well as strong radio-sensitizing ability to enhance X-ray-induced cancer cell killing, appeared to be a highly effective agent to improve *in vivo* antitumor efficacy of cancer radiation therapy.

Rapid clearance is a favorable behavior for nanomaterials to minimize their long-term body retention and potential toxicity.²⁸ To investigate the long-term clearance behaviors of W-GA-PEG CPNs, the time-dependent biodistribution study was carried out after *i.v.* injection of W-GA-PEG CPNs (40 mg/kg, two times higher than that used for enhanced radiotherapy) (Figure 5a). It was found that the retention of W-GA-PEG CPNs appeared to be relatively low in major organs including liver ($13.21 \pm 0.21 \text{ \%ID/g}$) and spleen ($9.01 \pm 0.47 \text{ \%ID/g}$) at 24 h *p.i.*, and further decreased over time, indicating the efficient clearance of W-GA-PEG CPNs from the mouse body. After two weeks, the liver and spleen retention of W decreased to be less than $0.2 \text{ \%ID/g}$, suggesting the nearly complete clearance of W-GA-PEG CPNs from the mouse body. To further investigate the clearance pathway of W-GA-PEG CPNs, the urine and feces of the mice post injection were collected at various time points. Only a small proportion of W-GA-PEG CPNs (about $1.69 \pm 0.46 \text{ \% ID}$) was eliminated through the binary pathway into feces in the first week, while up to $85.68 \pm 7.35 \text{ \%ID}$ of those was detected in the urine (Figure 5b). Our results strongly evidenced the rapid elimination of W-GA-PEG CPNs by passing the renal filtration, consistent with the above biodistribution analysis. Though the clearance rate of W-GA-PEG CPNs appeared to be slightly slower than certain types of ultrasmall nanodots coated with small molecules (*e.g.* glutathione),^{45,46}

possibly due to the PEG surface modification, W-GA-PEG CPNs still could be efficiently eliminated from mouse body *via* renal pathway.

Considering the substantial RES retention of W-GA-PEG CPNs and the fact that the majority of excreted W was found in the urine, we speculate that the excretion of such CPNs may occur *via* two different mechanism: (1) Ultrasmall CPNs may directly pass through glomerular filtration and excreted in the intact nanoparticle form, as observed in our TEM images of urine samples collected at 24 h *p.i.* (Supporting Figure S18). (2) For CPNs retained in the RES organs, those W-GA-PEG CPNs formed by non-covalent coordination interactions could be gradually degraded into ions and eliminated from body through renal pathway, similar to other types of CPNs as reported previously.^{47–49}

In order to find out whether W-GA-PEG CPNs would cause any long-term toxicity, we also carried out a series of experiments including blood chemistry, complete blood panel analysis, and histology examination on healthy mice after *i.v.* injection of W-GA-PEG CPNs (40 mg/kg). Major organs of treated mice were harvested at the 1st, 7th, and 30th post injection and sliced for hematoxylin and eosin (H&E) staining and histology analysis, in which no obvious tissue damages and adverse effects were found (Supporting Figure S19). Moreover, the parameters of blood chemistry and complete blood panel analysis in mice treated with W-GA-PEG CPNs showed no significant difference compared with those of control untreated mice (Figure 5c). All of our results suggest that W-GA-PEG CPNs upon systemic administration could be effectively excreted from the mouse body and would not result in any long-term side effect to the treated mice, promising for their *in vivo* biomedical applications.

Conclusion

In conclusion, ultra-small W-GA CPNs have been successfully synthesized *via* a simple one-step method based on the coordination interactions between W^{VI} and GA, and then modified by PEG to achieve excellent stability in various physiological solutions. Due to the high affinity between metal ions and the phenolic hydroxyl groups of GA, W-GA-PEG CPNs could efficiently label with ⁶⁴Cu²⁺ *via* a chelator-free method, enabling the tracking of W-GA-PEG CPNs after *i.v.* injection by PET imaging, which uncovers significant homing of those ultrasmall nanodots in the tumor, as well as their effective renal excretion. In the meanwhile, owing to the strong X-ray attenuation of W-GA-PEG CPNs, *in vivo* radiotherapy with greatly enhanced efficacy is achieved by W-GA-PEG to inhibit tumor growth upon X-ray radiation. Notably, after systemic administration, almost all of W-GA-PEG CPNs could rapidly excrete from treated mice *via* the renal system without any obvious long-term side effects. Our work illustrates a kind of renal-clearable coordination polymer nanodots useful for chelator-free radiolabeling, *in vivo* PET imaging of tumors, as well as enhanced radiotherapy with improved efficacy and no additional side effects. CPNs may thus be a promising type of nano-agent with great potential in clinical translation for cancer theranostics.

Experimental section

Material

All chemicals, unless otherwise specified, were obtained from Sigma-Aldrich and used as received. PEG polymers were purchased from Biomatrik Co., Ltd. (Jiaxing, China) with the molecular weight of 5 kDa. All aqueous solutions used in our experiments were prepared by using deionized water (18.2 MΩ.cm) obtained from the Milli-Q water purification system.

Synthesis of W-GA CPNs

In a typical procedure, 40 mg WCl_6 was dissolved in 8 mL water at room temperature under vigorous stirring. Then a PVP aqueous solution (1 mL, 66 mg/mL) was added into the above solution. After an hour, 10 mg GA dissolved in 1 mL water was dropwisely added into the mixture solution and reacted overnight. After that, the resulting solution was dialyzed against DI water for 24 h using a dialysis member with the molecular weight cut off (MWCO) at 8000–14000 Da. Finally, the obtained ultrasmall W-GA CPNs were stored at 4 °C for future use.

Surface modification of W-GA CPNs

For the PEGylation, 8 mL stock solution of W-GA CPNs were firstly mixed with 2 mL mPEG-NH₂ solution (MW = 5000, 10 mg/mL) at room temperature under vigorous stirring. After incubation for an hour, 4 mg N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) was added into the mixture, whose pH was adjusted to 7.4 by phosphate buffer. The reaction was allowed overnight, yielding a W-GA-PEG solution, which was purified by filtration with 10 kDa MWCO filters (Millipore) and stored at 4 °C for future use.

Characterization

A FEI Tecnai T20 transmission electron microscope was used for TEM imaging of W-GA CPNs. Power X-ray diffraction (XRD) was carried out by a PANalytical X-ray diffractometer. A PerkinElmer UV-vis-NIR spectrophotometer was used to obtained absorbance spectra. The dynamic light scattering (DLS) measurement was conducted *via* a Zetasizer Nano Z (Malvern). The morphology and size of W-GA CPNs was characterized by AFM (Veeco Inc.).

⁶⁴Cu-labeling and serum stability study

⁶⁴Cu was produced with an onsite cyclotron (GE PETtrace). 150 MBq of ⁶⁴CuCl₂ was diluted by sodium acetate buffer (pH 5.5) and mixed with W-GA-PEG CPNs (1 mg/mL) under constant shaking at 37 °C for 1 h. The labeling yield was obtained at different time points by thin layer chromatography (TLC). A PD-10 column was used to purify the obtained ⁶⁴Cu-W-GA-PEG using PBS as the mobile phase. For stability study, ⁶⁴Cu-W-GA-PEG was incubated with serum at 37 °C. After ultrafiltration with 10 kDa MWCO filters (Millipore), the released ⁶⁴Cu was collected for measurement by gamma counting.

In vitro experiments

Murine breast cancer 4T1 cells, murine colon cancer CT26 cells, human epithelial carcinoma HeLa cells, and mouse embryonic fibroblast NIH3T3 cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C under 5% CO₂. The MTT (Sigma-Aldrich) cell viability assay was conducted following the standard protocol.

For γ -H₂AX immunofluorescence staining, 4T1 cells seeded in 12-well plates were incubated with different concentrations of W-GA-PEG CPNs (0, 100 μ g/mL) for 6 h, and then subjected to irradiation of X-ray with the dose of 6 Gy. An hour later, the cells were fixed by 4% of paraformaldehyde for 10 min and washed with PBS twice. The γ -H₂AX immunofluorescence staining assay was then conducted following the standard protocol.⁵⁰ Confocal fluorescence images were taken by a Leica SP5 confocal fluorescence microscopy.

For clonogenic assay, 4T1 cells pre-seeded in 6-well plates were incubated with W-GA-PEG CPNs (0, 100 μ g/mL) at 37 °C for 24 h and then exposed to X-ray irradiation of different doses (0, 2, 4, and 6 Gy). After PBS washing for twice, the cells were further cultured with fresh culture medium for 8 days. Finally, the cells were fixed by methanol for 15 min and then stained by crystal violet for 30 min. The surviving fraction = (surviving colonies) / (cells seeded $\times$ plating efficiency).

Tumor model

Balb/c mice were housed following the protocols approved by University of Wisconsin Institutional Animal Care & Use Committee and Soochow University Laboratory Animal Center. Murine breast cancer 4T1 cells suspended in PBS were subcutaneously injected into the back of each Balb/c mouse to develop the tumor model.

PET imaging

4T1-tumor-bearing mice were intravenously injected with 5–10 MBq of ⁶⁴Cu-W-GA-PEG CPNs for PET imaging, performed by a microPET/microCT Inveon rodent model scanner (Siemens Medical Solutions USA, Inc.). The results were performed as described previously.⁵¹

In vivo radiotherapy

4T1-tumor-bearing mice were randomly divided into four groups (five mice per group): Group 1: PBS; Group 2: X-ray irradiation without nanoparticle (RT); Group 3: W-GA-PEG; Group 4: W-GA-PEG + RT. At 4 h post *i.v.* injection of W-GA-PEG with the dose of 20 mg/kg, mice in groups 2 and 4 were exposed to X-ray radiation at the dose of 6 Gy. The tumor volumes were measured after various treatments by a digital caliper. The tumor volume = length $\times$ width²/2. Mice with tumors larger than 1000 mm³ should be euthanized according to the standard animal protocol. Tumors of different groups were dissected on the second day post different treatments to make paraffin sections for further hematoxylin and eosin (H&E) staining following manufacturers' protocol.

Blood analysis and histology examination

After *i.v.* injection of W-GA-PEG CPNs (200 μ L, 20 mg/kg), Balb/c mice were randomly divided into three groups and sacrificed at the 1st, 7th, 30th day post injection. Another five untreated healthy mice were used as the control. Blood samples (~1 mL for each mouse) were drawn from mice for blood panel analysis and blood chemistry test examined by Shanghai Research Center of Biomodel Organism. Meanwhile, major organs were harvested from each mouse for H&E staining and histological examination.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Bardhan R, Lal S, Joshi A, Halas NJ. Theranostic Nanoshells: From Probe Design to Imaging and Treatment of Cancer. *Acc. Chem. Res.* 2011; 44:936–946. [PubMed: 21612199]
2. Yoo D, Lee JH, Shin TH, Cheon J. Theranostic Magnetic Nanoparticles. *Acc. Chem. Res.* 2011; 44:863–874. [PubMed: 21823593]
3. Cheng L, Wang C, Feng LZ, Yang K, Liu Z. Functional Nanomaterials for Phototherapies of Cancer. *Chem. Rev.* 2014; 114:10869–10939. [PubMed: 25260098]
4. Lee DE, Koo H, Sun IC, Ryu JH, Kim K, Kwon IC. Multifunctional Nanoparticles for Multimodal Imaging and Theragnosis. *Chem. Soc. Rev.* 2012; 41:2656–2672. [PubMed: 22189429]
5. Xie J, Liu G, Eden HS, Ai H, Chen XY. Surface-Engineered Magnetic Nanoparticle Platforms for Cancer Imaging and Therapy. *Acc. Chem. Res.* 2011; 44:883–892. [PubMed: 21548618]
6. Cobley CM, Chen JY, Cho EC, Wang LV, Xia YN. Gold Nanostructures: A Class of Multifunctional Materials for Biomedical Applications. *Chem. Soc. Rev.* 2011; 40:44–56. [PubMed: 20818451]
7. Chou LYT, Zagorovsky K, Chan WCW. DNA Assembly of Nanoparticle Superstructures for Controlled Biological Delivery and Elimination. *Nat. Nanotechnol.* 2014; 9:148–155. [PubMed: 24463361]
8. Al-Jamal WT, Kostarelos K. Liposomes: From a Clinically Established Drug Delivery System to a Nanoparticle Platform for Theranostic Nanomedicine. *Acc. Chem. Res.* 2011; 44:1094–1104. [PubMed: 21812415]
9. Sun C, Lee JSH, Zhang MQ. Magnetic Nanoparticles in MR Imaging and Drug Delivery. *Adv. Drug Deliver. Rev.* 2008; 60:1252–1265.
10. Xing HY, Bu WB, Zhang SJ, Zheng XP, Li M, Chen F, He QJ, Zhou LP, Peng WJ, Hua YQ, et al. Multifunctional Nanoprobes for Upconversion Fluorescence, MR and CT Trimodal Imaging. *Biomaterials.* 2012; 33:1079–1089. [PubMed: 22061493]
11. Li K, Liu B. Polymer-Encapsulated Organic Nanoparticles for Fluorescence and Photoacoustic Imaging. *Chem. Soc. Rev.* 2014; 43:6570–6597. [PubMed: 24792930]
12. Markman JL, Rekechenetskiy A, Holler E, Ljubimova JY. Nanomedicine Therapeutic Approaches to Overcome Cancer Drug Resistance. *Adv. Drug Deliver. Rev.* 2013; 65:1866–1879.

13. Liu JA, Bu WB, Pan LM, Shi JL. NIR-Triggered Anticancer Drug Delivery by Upconverting Nanoparticles with Integrated Azobenzene-Modified Mesoporous Silica. *Angew. Chem. Int. Ed.* 2013; 52:4375–4379.
14. Liu YT, Li K, Pan J, Liu B, Feng SS. Folic Acid Conjugated Nanoparticles of Mixed Lipid Monolayer Shell and Biodegradable Polymer Core for Targeted Delivery of Docetaxel. *Biomaterials.* 2010; 31:330–338. [PubMed: 19783040]
15. Al Zaki A, Joh D, Cheng ZL, De Barros ALB, Kao G, Dorsey J, Tsourkas A. Gold-Loaded Polymeric Micelles for Computed Tomography-Guided Radiation Therapy Treatment and Radiosensitization. *ACS Nano.* 2014; 8:104–112. [PubMed: 24377302]
16. Zhang XD, Chen J, Min Y, Park GB, Shen X, Song SS, Sun YM, Wang H, Long W, Xie JP, et al. Metabolizable Bi₂Se₃ Nanoplates: Biodistribution, Toxicity, and Uses for Cancer Radiation Therapy and Imaging. *Adv. Funct. Mater.* 2014; 24:1718–1729.
17. Zhang XD, Luo ZT, Chen J, Shen X, Song SS, Sun YM, Fan SJ, Fan FY, Leong DT, Xie JP. Ultrasmall Au_{10–12}(SG)_(10–12) Nanomolecules for High Tumor Specificity and Cancer Radiotherapy. *Adv. Mater.* 2014; 26:4565–4575. [PubMed: 24817169]
18. Dou Y, Guo YY, Li XD, Li X, Wang S, Wang L, Lv GX, Zhang XN, Wang HJ, Gong XQ, Chang J. Size-Tuning Ionization to Optimize Gold Nanoparticles for Simultaneous Enhanced CT Imaging and Radiotherapy. *ACS Nano.* 2016; 10:2536–2548. [PubMed: 26815933]
19. Xiao QF, Zheng XP, Bu WB, Ge WQ, Zhang SJ, Chen F, Xing HY, Ren QG, Fan WP, Zhao KL, et al. A Core/Satellite Multifunctional Nanotheranostic for *In Vivo* Imaging and Tumor Eradication by Radiation/Photothermal Synergistic Therapy. *J. Am. Chem. Soc.* 2013; 135:13041–13048. [PubMed: 23924214]
20. Nel A, Xia T, Madler L, Li N. Toxic Potential of Materials at the Nanolevel. *Science.* 2006; 311:622–627. [PubMed: 16456071]
21. Song XJ, Chen Q, Liu Z. Recent Advances in the Development of Organic Photothermal Nano-Agents. *Nano Res.* 2015; 8:340–354.
22. Liu JB, Yu MX, Zhou C, Zheng J. Renal Clearable Inorganic Nanoparticles: A New Frontier of Bionanotechnology. *Mater. Today.* 2013; 16:477–486.
23. Choi HS, Liu W, Misra P, Tanaka E, Zimmer JP, Ipe BI, Bawendi MG, Frangioni JV. Renal Clearance of Quantum Dots. *Nat. Biotechnol.* 2007; 25:1165–1170. [PubMed: 17891134]
24. Longmire M, Choyke PL, Kobayashi H. Clearance Properties of Nano-Sized Particles and Molecules as Imaging Agents: Considerations and Caveats. *Nanomedicine-UK.* 2008; 3:703–717.
25. Yu MX, Liu JB, Ning XH, Zheng J. High-Contrast Noninvasive Imaging of Kidney Clearance Kinetics Enabled by Renal Clearable Nanofluorophores. *Angew. Chem. Int. Ed.* 2015; 54:15434–15438.
26. Yu MX, Zhou JC, Du BJ, Ning XH, Authement C, Gandee L, Kapur P, Hsieh JT, Zheng J. Noninvasive Staging of Kidney Dysfunction Enabled by Renal-Clearable Luminescent Gold Nanoparticles. *Angew. Chem. Int. Ed.* 2016; 55:2787–2791.
27. Choi HS, Liu WH, Liu FB, Nasr K, Misra P, Bawendi MG, Frangioni JV. Design Considerations for Tumour-Targeted Nanoparticles. *Nat. Nanotechnol.* 2010; 5:42–47. [PubMed: 19893516]
28. Choi HS, Frangioni JV. Nanoparticles for Biomedical Imaging: Fundamentals of Clinical Translation. *Mol. Imaging.* 2010; 9:291–310. [PubMed: 21084027]
29. Mao FX, Wen L, Sun CX, Zhang SH, Wang GL, Zeng JF, Wang Y, Ma JM, Gao MY, Li Z. Ultrasmall Biocompatible Bi₂Se₃ Nanodots for Multimodal Imaging-Guided Synergistic Radiophotothermal Therapy against Cancer. *ACS Nano.* 2016; 10:11145–11155. [PubMed: 28024338]
30. Liu T, Chao Y, Gao M, Liang C, Chen Q, Song GS, Cheng L, Liu Z. Ultra-Small MoS₂ Nanodots with Rapid Body Clearance for Photothermal Cancer Therapy. *Nano Res.* 2016; 9:3003–3017.
31. Oh M, Mirkin CA. Chemically Tailorable Colloidal Particles from Infinite Coordination Polymers. *Nature.* 2005; 438:651–654. [PubMed: 16319888]
32. Lin WB, Rieter WJ, Taylor KML. Modular Synthesis of Functional Nanoscale Coordination Polymers. *Angew. Chem. Int. Ed.* 2009; 48:650–658.
33. Liu DM, Poon C, Lu KD, He CB, Lin WB. Self-Assembled Nanoscale Coordination Polymers with Trigger Release Properties for Effective Anticancer Therapy. *Nat. Commun.* 2014; 5:1–11.

34. Horcajada P, Chalati T, Serre C, Gillet B, Sebrie C, Baati T, Eubank JF, Heurtaux D, Clayette P, Kreuz C, et al. Porous Metal-Organic-Framework Nanoscale Carriers as a Potential Platform for Drug Delivery and Imaging. *Nat. Mater.* 2010; 9:172–178. [PubMed: 20010827]
35. Della Rocca J, Liu DM, Lin WB. Nanoscale Metal-Organic Frameworks for Biomedical Imaging and Drug Delivery. *Acc. Chem. Res.* 2011; 44:957–968. [PubMed: 21648429]
36. Liu FY, He XX, Chen HD, Zhang JP, Zhang HM, Wang ZX. Gram-Scale Synthesis of Coordination Polymer Nanodots with Renal Clearance Properties for Cancer Theranostic Applications. *Nat. Commun.* 2015; 6:1–9.
37. Jin R, Li H, Deng J-F. Selective Oxidation of Cyclopentene to Glutaraldehyde by H₂O₂ over the WO₃/SiO₂ Catalyst. *J. Catal.* 2001; 203:75–81.
38. Di Fagagna FD, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, von Zglinicki T, Saretzki G, Carter NP, Jackson SP. A DNA Damage Checkpoint Response in Telomere-Initiated Senescence. *Nature.* 2003; 426:194–198. [PubMed: 14608368]
39. Bailey DL, Townsend DW, Valk PE, Maisey MN. Positron Emission Tomography: Basic Sciences. *Radiology.* 2006; 241:45–45.
40. Chen DQ, Yang DZ, Dougherty CA, Lu WF, Wu HW, He XR, Cai T, Van Dort ME, Ross BD, Hong H. *In Vivo* Targeting and Positron Emission Tomography Imaging of Tumor with Intrinsically Radioactive Metal-Organic Frameworks Nanomaterials. *ACS Nano.* 2017; 11:4315–4327. [PubMed: 28345871]
41. Chen F, Hong H, Zhang Y, Valdovinos HF, Shi SX, Kwon GS, Theuer CP, Barnhart TE, Cai WB. *In Vivo* Tumor Targeting and Image-Guided Drug Delivery with Antibody-Conjugated, Radio labeled Mesoporous Silica Nanoparticles. *ACS Nano.* 2013; 7:9027–9039. [PubMed: 24083623]
42. Ellison P, Chen F, Lewis C, Hong H, Meyerand M, Barnhart T, DeJesus O, Cai WB. Chelator-Free Synthesis of a Dual-Modality PET/MRI Agent. *J. Nucl. Med.* 2014; 55:62–73.
43. Chen F, Ellison PA, Lewis CM, Hong H, Zhang Y, Shi SX, Hernandez R, Meyerand ME, Barnhart TE, Cai WB. Chelator-Free Synthesis of a Dual-Modality PET/MRI Agent. *Angew. Chem. Int. Ed.* 2013; 52:13319–13323.
44. Zhou M, Zhang R, Huang MA, Lu W, Song SL, Melancon MP, Tian M, Liang D, Li C. A Chelator-Free Multifunctional [Cu-64] CuS Nanoparticle Platform for Simultaneous Micro-PET/CT Imaging and Photothermal Ablation Therapy. *J. Am. Chem. Soc.* 2010; 132:15351–15358. [PubMed: 20942456]
45. Huang HY, Hernandez R, Geng JM, Sun HT, Song WT, Chen F, Graves SA, Nickles RJ, Cheng C, Cai WB, Lovell JF. A Porphyrin-PEG Polymer with Rapid Renal Clearance. *Biomaterials.* 2016; 76:25–32. [PubMed: 26517562]
46. Yang SY, Sun SS, Zhou C, Hao GY, Liu JB, Ramezani S, Yu MX, Sun XK, Zheng J. Renal Clearance and Degradation of Glutathione-Coated Copper Nanoparticles. *Bioconjugate Chem.* 2015; 26:511–519.
47. Mehravi B, Ahmadi M, Amanlou M, Mostaar A, Ardestani MS, Ghalandarlaki N. Conjugation of Glucosamine with Gd³⁺-based Nanoporous Silica Using a Heterobifunctional ANB-NOS Crosslinker for Imaging of Cancer Cells. *Int. J. Nanomed.* 2013; 8:3383.
48. Yang Y, Liu JJ, Liang C, Feng LZ, Fu TT, Dong ZL, Chao Y, Li YG, Lu G, Chen MW, Liu Z. Nanoscale Metal-Organic Particles with Rapid Clearance for Magnetic Resonance Imaging-Guided Photothermal Therapy. *ACS Nano.* 2016; 10:2774–2781. [PubMed: 26799993]
49. Liu JJ, Yang Y, Zhu WW, Yi X, Dong ZL, Xu XN, Chen MW, Yang K, Lu G, Jiang LX, et al. Nanoscale Metal-Organic Frameworks for Combined Photodynamic & Radiation Therapy in Cancer Treatment. *Biomaterials.* 2016; 97:1–9. [PubMed: 27155362]
50. Song GS, Liang C, Gong H, Li MF, Zheng XC, Cheng L, Yang K, Jiang XQ, Liu Z. Core-Shell MnSe@Bi₂Se₃ Fabricated *via* a Cation Exchange Method as Novel Nanotheranostics for Multimodal Imaging and Synergistic Thermoradiotherapy. *Adv. Mater.* 2015; 27:6110–6117. [PubMed: 26331476]
51. Cai WB, Chen XY. Preparation of Peptide-Conjugated Quantum Dots for Tumor Vasculature-Targeted Imaging. *Nat. Protoc.* 2008; 3:89–96. [PubMed: 18193025]

Figure 1.

Synthesis and characterization of W-GA CPNs. (a) Schematic illustration for the synthesis and structure of W-GA-PEG CPNs. (b) A TEM image of as-synthesized W-GA CPNs. Inset: The photo of W-GA CPNs after centrifugal filtration through a 100-kDa molecular weight cut-off filter (Millipore) and TEM measured diameter distribution of as-synthesized W-GA CPNs. (c) An AFM image of W-GA CPNs. Inset: The height of as-synthesized W-GA CPNs measured by AFM. (d) XPS spectra of W 4f orbits of W-GA CPNs. (e) W L₃-edge X-ray absorption near edge structure (XANES) spectra of the W-GA CPNs and model. (f) FTIR spectra of GA and as-synthesized W-GA CPNs. (g) Hydrodynamic diameters of W-GA-PEG CPNs in various physiological solutions. Inset: A photograph of W-GA-PEG CPNs in these three solutions after incubation for 24 h.

Figure 2.

In vitro enhanced radiotherapy with W-GA-PEG CPNs. **(a)** Relative cell viabilities of murine breast cancer (4T1) cells, murine colon cancer (CT26) cells, human epithelial carcinoma (HeLa) cells, and mouse embryonic fibroblast (NIH3T3) cells after incubation with various concentrations of W-GA-PEG CPNs for 24 h. **(b)** Clonogenic survival assay of 4T1 cells treated with W-GA-PEG CPNs exposed to various radiation doses at 0, 2, 4, and 6 Gy. **(c)** Confocal images of double-strand DNA breaks marked by $\gamma\text{-H2AX}$ in 4T1 cells treated with PBS, RT, W-GA-PEG, and W-GA-PEG + RT. The X-ray irradiation dose was 6 Gy.

Figure 3.

^{64}Cu -labeled W-GA-PEG CPNs and *in vivo* PET imaging. **(a)** Schematic illustration of ^{64}Cu -labeled W-GA-PEG CPNs *via* the chelator-free manner. **(b)** Radiolabeling efficiency of ^{64}Cu -W-GA-PEG after incubation for various time points. **(c)** Radiolabeling stability of ^{64}Cu -labeled W-GA-PEG in serum. **(d)** *In vivo* PET images of 4T1-tumor-bearing mice after *i.v.* injection of ^{64}Cu -W-GA-PEG CPNs taken at different time points. The tumors are indicated with yellow arrows. **(e)** Quantification of ^{64}Cu -W-GA-PEG uptake in the liver, kidney, tumor, bladder and muscle at various time points *p.i.* **(f)** Biodistribution of W-GA-PEG CPNs at various time points post *i.v.* injection measured by ICP-AES.

Figure 4.

In vivo enhanced radiotherapy with W-GA-PEG CPNs. (a) Tumor growth curves of different groups (five mice per group) after various treatments. The irradiation dose of X-ray (RT) was 6 Gy and the injection dose of W-GA-PEG CPNs was 20 mg/kg. (b) Survival curves of mice after various treatments as indicated in (a). (c) H&E stained tumor slices from various groups 24 h after receiving different treatments.

Figure 5. Biodistribution and *in vivo* long-term toxicity of W-GA-PEG CPNs. (a) Biodistribution of W-GA-PEG CPNs after *i.v.* injection in healthy mice measured at different time points by ICP-AES. (b) The W levels in urine and feces of healthy mice after *i.v.* administration of W-GA-PEG CPNs (dose = 40 mg/kg) collected at different intervals. (c) Blood biochemistry / complete blood panel analysis data of healthy mice after *i.v.* injection of PBS or W-GA-PEG CPNs (40 mg/kg) collected at 1, 7, 30 days post injection. Reference ranges of hematology data of healthy female Balb/c mice.⁴⁹