Core–Satellite Polydopamine–Gadolinium-Metallofullerene Nanotheranostics for Multimodal Imaging Guided Combination Cancer Therapy

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Integration of magnetic resonance imaging (MRI) and other imaging modalities is promising to furnish complementary information for accurate cancer diagnosis and imaging-guided therapy. However, most gadolinium (Gd)–chelator MR contrast agents are limited by their relatively low relaxivity and high risk of released-Gd-ions-associated toxicity. Herein, a radionuclide-64Cu-labeled doxorubicin-loaded polydopamine (PDA)–gadolinium-metallofullerene core–satellite nanotheranostic agent (denoted as CDPGM) is developed for MR/photoacoustic (PA)/positron emission tomography (PET) multimodal imaging-guided combination cancer therapy. In this system, the near-infrared (NIR)-absorbing PDA acts as a platform for the assembly of different moieties; Gd₃N@C₈₀, a kind of gadolinium metallofullerene with three Gd ions in one carbon cage, acts as a satellite anchoring on the surface of PDA. The as-prepared CDPGM NPs show good biocompatibility, strong NIR absorption, high relaxivity ($r_1 = 14.06 \text{ mM}^{-1} \text{s}^{-1}$), low risk of release of Gd ions, and NIR-triggered drug release. In vivo MR/PA/PET multimodal imaging confirms effective tumor accumulation of the CDPGM NPs. Moreover, upon NIR laser irradiation, the tumor is completely eliminated with combined chemophotothermal therapy. These results suggest that the CDPGM NPs hold great promise for cancer theranostics.

Multimodal imaging is promising to furnish complementary information, such as anatomic, physiologic, molecular, and genomic information, for accurate diagnosis, monitoring therapy response, guiding drug discovery/development, and so on. Among different imaging modalities, magnetic resonance imaging (MRI) is one of the most powerful and noninvasive techniques because it can provide images with excellent spatial resolution without ionizing radiation. Photoacoustic imaging (PAI), which is a new promising biomedical imaging modality based on the photoacoustic effect, combines the high selectivity of optical imaging and the deep-tissue penetration of ultrasonic imaging and can provide molecular information in high sensitivity. Positron emission tomography (PET) is a widely used clinical imaging technique with high sensitivity and quantitative accuracy. Therefore, the integration of MRI, PAI, and PET trimodal imaging would offer high resolution, selectivity, and sensitivity.

It is well known that MRI suffers from low sensitivity. To improve the sensitivity of MRI in practical diagnosis, various MR contrast agents have been developed to alter the longitudinal ($T_1$) or transverse ($T_2$) relaxation times of surrounding water protons and increase the contrast of the target from the background. Gadolinium (Gd)–chelator complexes, such as Magnevist (Gd–DTPA), are the most widely used MR contrast agents in over 25–30% of clinical MRI procedures. However,
most of these Gd–chelator agents are limited by their relatively low relaxivity and high risk of released-Gd-ion-associated toxicity.\[8\] Recently, Gd-containing metallofullerenes (e.g., Gd@C_{60}, Gd@C_{82}, and Gd_{3}N@C_{80}) have been employed as MR contrast agents with efficient MRI performance and high safety.\[9\] On the one hand, these metallofullerenes exhibit much higher (10–40 times) relaxivity than most commercial Gd–chelator agents; on the other hand, the toxic Gd ions are confined inside a robust carbon cage, thus their leakage is suppressed and Gd-ion-associated toxicity is decreased. Therefore, the development of Gd-containing metallofullerenes-based multimodal imaging contrast agent is highly desirable for optimizing MRI and other imaging modalities.

For cancer therapy, chemotherapy is used as a common strategy, but limited by the inevitable problems such as low treatment efficacy and severe side effects.\[10\] To address these problems, strategies that combine chemotherapy with other therapeutic approaches have been actively pursued to improve the treatment efficacy.\[11\] Among those approaches, photothermal therapy (PTT), which employs photothermal conversion agents (PTCAs) and near-infrared (NIR) light-induced photothermal effect to ablate cancer cells/tissues, shows great combination therapeutic effect when applied together with chemotherapy.\[11\] To date, a series of NIR light-absorbing conducting and semiconducting polymers with conjugated structures have been developed as PTCAs for PTT.\[11\] Particularly, polydopamine (PDA), which has a melanin-like chemical structure, has been verified to be an excellent PTCA due to its good biocompatibility/biodegradability and excellent photothermal stability.\[11\] Furthermore, during the synthetic process of PDA nanoparticles (NPs), many functional moieties, such as drugs, photosensitizers, contrast agents, and so on, can be integrated for multifunctional theranostics.\[11\]

Here, we developed a radionuclide-\[^{64}\text{Cu}\]-labeled doxorubicin (DOX)-loaded polydopamine (PDA)–gadolinium-metallofullerene (Gd_{3}N@C_{80}) core–satellite nanotheranostics (denoted as CDPGM) for multimodal imaging guided combination cancer therapy (Figure 1). In this system, PDA NP not only acted as a core platform for the assembly of different moieties, but also served as a NIR-absorbing PTCA with good biocompatibility and photothermal stability for PTT and PAI. Gd_{3}N@C_{80}, with three Gd ions in one carbon cage, plays as the satellite anchoring on the surface of PDA for MRI.\[6\] The radionuclide \[^{64}\text{Cu}\] was coupled with CDPGM NPs through the strong affinity of copper ions to catechol groups of PDA for PET imaging. Moreover, considering abundant \(\pi\)-conjugated structures of PDA, DOX as a model antitumor drug, can be loaded into PDA NPs through \(\pi\)-\(\pi\) stacking and hydrogen-bonding interactions. The as-prepared CDPGM NPs demonstrate three distinct features: i) the PDA–gadolinium-metallofullerene core–satellite structure of the CDPGM NPs permits fast exchange of water and thus has high relaxivity and low risk of release of Gd ions; ii) MR/PA/PET trimodal imaging allows more accurate diagnosis and imaging-guided therapy than single modality alone; iii) high photothermal performance of PDA and pH1-/NIR-triggered drug release lead to effective chemo-photothermal combination therapy.

The fabrication process of CDPGM NPs is shown in Figure S1 (Supporting Information). The amino-functionalized Gd_{3}N@C_{80} (Gd_{3}N@C_{80}-NH\textsubscript{2}) was prepared and purified by a reported procedure.\[6\] The as-prepared functionalized Gd_{3}N@C_{80}-NH\textsubscript{2} can be well-dispersed in water with a diameter of 5–20 nm and height around 3 nm (Figure S2, Supporting Information). This result is in agreement with the literature.\[6\] PDA NPs were prepared by self-polymerization of dopamine monomer in a water/ethanol/ammonia mixture.\[11\] Then Gd_{3}N@C_{80}-NH\textsubscript{2} and poly(ethylene glycol) (PEG) were conjugated to the surface of PDA NPs by \(\pi\)-\(\pi\) interaction and the reaction between amino groups and quinone structures. The Gd concentration of PDA–gadolinium-metallofullerene (PGM) NPs was determined by using inductively coupled plasma mass spectrometry (ICP-MS), which demonstrated that the Gd_{3}N@C_{80}-NH\textsubscript{2} was successfully conjugated with PDA NPs (Figure S3, Supporting Information). Nuclear magnetic resonance (\(^1\text{H NMR}\)) characterization also demonstrated that PEG chains were coupled onto PDA NPs (Figure S4, Supporting Information). The size of PGM NPs was detected by transmission electron microscopy (TEM) and dynamic light scattering (DLS). As shown in Figure 2a, PGM NPs showed well-defined spherical structure with the diameter around 100 nm. The hydrodynamic diameter of PGM NPs measured by DLS was 120.8 ± 22.0 nm (Figure S5, Supporting Information), and showed good colloidal stability for at least three weeks (Figure S6, Supporting Information). Both PDA NPs and PGM NPs exhibited broad absorption ranging from ultraviolet–visible (UV–vis) to NIR region (Figure S7, Supporting Information). The absorption spectra of PGM NPs in Figure S8 (Supporting Information) demonstrated the concentration-dependent NIR absorbance increase, with a good linear relationship of optical density at 808 nm versus its concentration (mg L\(^{-1}\)): \(Y = 0.0034X – 0.0062, \ R = 0.999\) (Figure S9, Supporting Information).

To investigate the MRI capability, the longitudinal proton relaxation times (\(T\textsubscript{1}\)) of PGM NPs and commercial Gd–DTPA were compared under the same condition by using a 7 T MR scanner. As shown in the inset of Figure 2b, the MR signal...
intensity increased with the increase of Gd concentrations. The calculated $r_1$ of PGM NPs is 14.06 mM$^{-1}$s$^{-1}$, which is over threefold higher than that of Gd–DTPA (4.35 mM$^{-1}$s$^{-1}$). This high $r_1$ value of PGM NPs is attributed to the great MRI performance of gadolinium metallofullerene. Furthermore, PGM NPs showed high stability without obvious release of Gd ions (Figure S10, Supporting Information). This excellent stability of PGM NPs may be attributed to the unique structure of Gd$_3$N@C$_{80}$ that confines the toxic Gd ions inside an inert and robust carbon cage. [9c] These results suggested that PGM NPs could be a promising MR contrast agent for MRI.

To evaluate the photothermal properties of PGM NPs, their aqueous solutions with different concentrations were exposed to a NIR laser (808 nm, 1.0 W cm$^{-2}$, 5 min), and the temperatures of solutions were monitored. As shown in Figure 2d, even at a low concentration (80 µg mL$^{-1}$), the temperature of PGM NP solution increased rapidly, with a temperature change ($\Delta T$) of 29.6 °C in 5 min, which demonstrated great NIR light-induced thermal effect of the PGM NPs. In contrast, pure water showed negligible temperature change upon NIR laser irradiation. The photothermal conversion efficiency ($\eta$) of the PGM NPs was determined to be 39.78% (Figure S11, Supporting Information), which is significantly higher than that of gold nanorods (27.12%) (Figure S12, Supporting Information). To evaluate the photothermal stability, the temperature changes of PGM NPs suspension in five laser on-off cycles were monitored. As shown in Figure 2e, even in the fifth cycle of laser exposure, the solution temperature can still rise to the same level. The absorption of PGM NPs almost remained unchanged after laser irradiation (Figure S13, Supporting Information), suggesting good photothermal stability of PGM NPs.

A clinically used chemotherapy drug, DOX, was loaded into PGM NPs through $\pi$–$\pi$ stacking and hydrogen-bonding interactions, to obtain DOX-loaded PGM (DPGM) NPs. As

Figure 2. a) TEM image of PGM NPs. b) Longitudinal relaxation rates ($1/T_1$) of PGM NPs and Gd–DTPA as a function of Gd concentration ($\times 10^{-3}$ M). Inset: $T_1$-weighted MR image of PGM NPs at different Gd concentrations. c) PA signals at 808 nm of PGM NPs as a function of concentration. d) Photothermal heating curves of PGM NPs aqueous solution upon 808 nm laser irradiation (1.0 W cm$^{-2}$). e) Temperature change of PGM NPs aqueous solution under 808 nm laser irradiation (1.0 W cm$^{-2}$) for five laser on-off cycles. f) UV–vis–NIR absorption spectra of DOX and DPGM NPs aqueous solution. g) The DOX loading efficiency at different DOX: PGM ratios ($n = 3$). h) In vitro drug-release profiles of DPGM NPs in media with different pH values ($n = 3$). i) NIR-triggered drug release of DPGM NPs aqueous solution ($n = 3$).
shown in the absorption spectra (Figure 2f), compared with PGM NPs, a typical peak at 480 nm was observed in the spectra of DPGM NPs, indicating successful loading of DOX. With the increasing amount of feeding drug, the peak intensity at 480 nm gradually increased, suggesting the increased drug loading content. As shown in Figure 2g, the maximal drug loading amount was ≈40%, demonstrating great drug loading capability of PGM NPs. The stimuli-responsive drug-release behaviors of DPGM NPs were then investigated with the application of diverse stimuli including pH and NIR laser irradiation. To study the pH-responsive drug release, DPGM NPs were dispersed in phosphate buffered solution (PBS) at different pH values (7.4 and 6.0) and the released DOX was collected and analyzed by UV–vis calibration curve (Figure S14, Supporting Information). As shown in Figure 2h, at pH 7.4, only 17.85% of DOX was released from DPGM NPs in 24 h, indicating good stability.
of DPGM NPs under neutral condition. In contrast, due to the increased solubility of protonated DOX, about 50.06% of DOX was released at pH 6.0. Then the NIR-triggered drug release was also investigated. As shown in Figure 2i, under the irradiation of 808 nm NIR light (1.0 W cm\(^{-2}\), 5 min), a burst release of DOX was observed at both pH 7.4 and 6.0, with about 27.3% and 46.7% DOX loss in 180 min, respectively. This pH/NIR dual stimuli-responsive drug-release behavior of DPGM NPs made them promising for controlled chemotherapy.

The cell cytotoxicity and in vitro chemo-photothermal combination therapy of DPGM NPs were then evaluated on U87MG cells by a MTT assay. As shown in Figure 3a, PGM NPs exhibited negligible toxicity to cells even at a high concentration (200 mg L\(^{-1}\)) after incubation for 24 h. However, when an 808 nm NIR irradiation (1.0 W cm\(^{-2}\), 5 min) was applied, the viability of U87MG cells decreased significantly as the sample concentration increased, with a cell viability of less than 10% at a concentration of 100 mg L\(^{-1}\). Meanwhile, the live/dead cells were co-stained with calcein AM (green fluorescence) and propidium iodide (PI, red fluorescence). As shown in Figure S15 (Supporting Information), the calcein AM and PI co-staining results also confirmed the effective photothermal therapy effect of PGM NPs with NIR irradiation, which were consistent with the MTT results. These results demonstrated that PGM NPs are an effective photothermal agent for NIR-induced tumor therapy. The NIR-enhanced cellular uptake of DPGM NPs was investigated on U87MG cells. As shown in Figure 3b, DOX fluorescence intensity in cells incubated with free DOX with or without NIR laser irradiation showed no obvious difference. However, for cells incubated with DPGM NPs with NIR laser irradiation, higher fluorescence intensity was observed when compared to that without laser irradiation. This result may be attributed to mild hyperthermia-enhanced cellular uptake and NIR-triggered drug release. Then we investigated the antitumor activities of free DOX and DPGM NPs against U87MG cells in vitro. As shown in Figure S16 (Supporting Information), after 24 h incubation, dose-dependent antitumor activities of both free DOX and DPGM NPs were observed. To study the chemo-photothermal combination therapy of the DPGM NPs, free DOX, PGM NPs, and DPGM NPs were incubated with U87MG cells for 4 h. After the incubation, the cells were exposed to 808 nm NIR laser irradiation (1.0 W cm\(^{-2}\), 5 min) and then further incubated for 20 h. As shown in Figure 3c, compared to the monotherapy, the combination therapy showed significantly improved antitumor effect with a cell viability of only 18.6%. These results suggested that the DPGM NPs can achieve great combination antitumor effect.

To demonstrate the multimodal imaging capacity of PGM NPs, we performed \(T_1\)-weighted MRI and PAI on U87MG tumor-bearing mice. As shown in Figure 4a, the tumor area showed a relatively low MR signal intensity before injection. After intravenous

**Figure 4.** In vivo multimodal imaging. a) MR images and b) signal-to-noise ratio (SNR) changes of U87MG-tumor-bearing mice pre- and postintravenous injection of PGM NPs. c) PA images and d) PA signal intensity changes of U87MG-tumor-bearing mice pre- and postintravenous injection of PGM NPs. e) PET images of U87MG tumor-bearing mice after intravenous injection of CPGM NPs. f) Time-dependent tumor uptake of CPGM NPs.
injection of PGM NPs, the tumor MR signal intensity gradually increased over time (Figure 4a). The changes of signal-to-noise ratio (SNR\text{post}/SNR\text{pre}) at the tumor were quantitatively calculated, showing 1.21- and 1.53-fold increase at 4 and 24 h, respectively (Figure 4b). PA images of tumor region were acquired at preinjection and 2, 4, 6, 24, 32, 48, and 72 h postinjection. As shown in Figure 4c,d, the PA signal intensity of tumor region gradually increased and reached the maximum value at 24 h postinjection, suggesting effective tumor accumulation of PGM NPs. After 24 h, the tumor PA signal intensity decreased slightly. As the catechol groups on the surface of PGM NPs have affinity for metal ions, here, radionuclide $^{64}$Cu was labeled to PGM NPs for PET imaging. The labeling efficiency of CPGM NPs evaluated by instant thin layer chromatography (iTLC) was about 100%. The stability study showed that the CPGM NPs were still stable without obvious free copper leakage after laser irradiation (Figure S17, Supporting Information). Then the in vivo delivery and biodistribution of CPGM NPs was evaluated by PET. As shown in the decay-corrected PET images (Figure 4e), a high tumor-to-background contrast was observed in the CPGM-NPs-treated U87MG tumor-bearing nude mice. The tumor uptake efficiency of CPGM NPs was measured by using a quantitative 3D volume-of-interest analysis method. As shown in Figure 4f, the tumor uptake of CPGM NPs gradually increased within 24 h, after which it decreased. At 24 and 48 h postinjection, the mice were sacrificed and the major organs were collected for direct tissue sampling biodistribution study. As shown in Figure S18 (Supporting Information), 5.34% and 3.99% ID g$^{-1}$ of tumor uptakes were achieved at 24 and 48 h postinjection, respectively. These results suggest that the CPGM NPs can be used for simultaneous MR/PA/PET multimodal imaging.

Then the in vivo chemo-photothermal combination therapy was also carried out on the U87MG tumor-bearing mice. As shown in Figure 5a,b, upon 10 min of 808 nm laser irradiation, the tumor temperature did not show significant change for

**Figure 5.** In vivo combination therapy of DPGM NPs on U87MG tumor-bearing mice. a) Thermographic images of mice treated with an NIR laser (808 nm, 0.8 W cm$^{-2}$, 10 min) at 24 h postinjection of samples. b) Temperature changes of tumor regions. c) Relative tumor volume of the mice after different treatments. **$p < 0.01$, ***$p < 0.001$. d) Representative photos of U87MG tumor-bearing mice after different treatments. e) Survival curves of the mice after different treatments. f) H&E staining images of tumor sections.
mice injected with PBS. In contrast, the tumor temperature increased rapidly and maintained at about 46 °C for mice injected with PGM NPs and DPGM NPs. In the following three weeks, the tumor volumes were monitored every 2 d. As shown in Figure 5c, the PBS + NIR laser treatment group showed almost no effect on tumor growth; however, both chemotherapy and PTT groups slowed the growth of tumors. Particularly, in the combination therapy group, the tumor was completely eradicated without regrowth or recurrence (Figure 5d), and their survival was greatly prolonged (Figure 5e). A hematoxylin and eosin (H&E) staining analysis of tumors was also performed to further determine the therapeutic effect of different groups (Figure 5f). The tumors treated with combination therapy showed much higher damage than those in the other groups. This improved therapeutic effect of the combination therapy may be attributed to hyperthermia effect and NIR-triggered drug release. As an indicator of systemic toxicity, mouse body weights were also measured. As shown in Figure S19 (Supporting Information), only free-DOX-treated mice showed slight body weight decrease in the early several days, while the other groups showed no noticeable body weight change. At last, the long-term in vivo toxicity of PGM NPs was investigated. Major organs (heart, liver, spleen, lungs, and kidneys) were collected at 40 d postinjection. As shown in Figure S20 (Supporting Information), there was no obvious organ damage in the PGM group, demonstrating good biocompatibility of the PGM NPs.

In summary, we have developed a multifunctional theranostic agent based on 64Cu-labeled doxorubicin-loaded polydopamine–gadolinium-metallofulleren core–satellite nanoparticles (CDPGM NPs) for MR/PA/PTT multimodal imaging-guided chemo-photothermal combination therapy. In this system, PDA acted as a platform for the assembling of different moieties. The as-prepared nanotheranostics showed good biocompatibility, strong NIR absorption, high relaxivity ($r_1 = 14.06$ mM$^{-1}$ s$^{-1}$), low risk of Gd-ion release, and NIR-triggered drug release. In vivo MR/PA/PTT multimodal imaging confirmed effective tumor accumulation of the CDPGM NPs. Moreover, upon NIR laser irradiation, the tumor was completely eliminated with combined chemo-photothermal therapy. These results suggest that the CDPGM NPs hold great promise for cancer theranostics. This work may encourage further explorations of metallofullerene-based nano-theranostics for applications in multimodal imaging guided cancer therapy.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

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