Porphyric Metal–Organic Frameworks Coated Gold Nanorods as a Versatile Nanoplatform for Combined Photodynamic/Photothermal/Chemotherapy of Tumor

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In this paper, a simple, but effective method is reported to construct the core−shell gold nanorod@metal−organic frameworks (AuNR@MOFs) as a multifunctional theranostic platform by using functionalized AuNRs as seed crystal for the growth of porphyrinic MOFs on the surface of AuNR. Such a delicate tunable core−shell composite not only possesses the improved drug loading efficiency, near-infrared light-trigger drug release, and fluorescence imaging, but also can produce reactive oxygen species as well as photothermal activity to achieve combined cancer therapy. It is further demonstrated that the camptothecin loaded AuNR@MOFs show distinctively synergistic efficiency for damaging the cancer cell in vitro and inhibiting the tumor growth and metastasis in vivo. The development of this high-performance incorporated nanostructure will provide more perspectives in the design of versatile nanomaterials for biomedical applications.

1. Introduction

Cancer is considered to be the most serious disease and the development of efficient cancer treatment is urgent. The conventional cancer treatments, such as chemotherapy, radiotherapy, and surgery, have been unable to satisfy the demand for cancer therapy owing to the severe side effect and low therapeutic efficiency.[2] To solve these problems, researchers have paid much attention to developing combined cancer treatments, which have presented considerable potential in overcoming multi-drug resistance, improving tumoricidal effect and minimizing the invasive damage.[2] The integration of different anticancer modalities, such as chemotherapy, photothermal therapy (PTT) and photodynamic therapy (PDT), into a nanoplatform with unique physicochemical properties has exhibited great potential to achieve high anticancer efficacy and low risk of recurrence.[3]

Although combined therapy of PTT/PDT, PDT/chemotherapy, and PTT/chemotherapy have been reported, nanoplatform-based PDT/PTT/chemotherapy remains in the early stages, which is important to lower laser power and reduce drug dosage for combination therapy.[4] Recently, some multifunctional nanomaterials such as mesoporous silica-coated gold nanorods (AuNRs) black phosphorus, and upconversion nanoparticles based PDT/PTT/chemotherapy have been explored, exhibiting the outstanding anticancer performance.[5] However, to develop a simple, but effective strategy to construct multifunctional nanoplatform with higher loading capacity (LC) for PDT/PTT/chemotherapy is still a great challenge.

Metal–organic frameworks (MOFs), an emerging class of porous materials, have received significant attention for their applications in gas storage, catalysis, and drug delivery.[6] Due to tunable inorganic building blocks and organic linkers, MOFs can not only integrate photosensitizers into periodic arrays, but also possess large pore sizes and high surface areas for drug encapsulation.[7] Incorporating metal nanostructure within MOFs to obtain the composites with collective properties and improved performance attracts much research attention. Firstly, due to the MOFs shell coating, the aggregation and migration of the nanostructures with high surface energy and small size can be avoided, so that the chemical activity and stability of the nanostructure cores were preserved.[8] Additionally, the advantages of both the nanostructures and the MOFs shells can be effectively combined and novel chemical and physical properties that are not possible from single-component nanostructure may be accessed. Several studies have reported that selective nanostructure capping surfactants or polymers, such as hexadecyl trimethyl ammonium bromide (CTAB), polyvinylpyrrolidone (PVP), and polydopamine (PDA), can mediate the growth of MOFs shell on metal nanostructures to form core−shell nanohybrids.[9] Although there are many studies on core−shell nanostructure@MOFs, the integration of nanostructures in functional MOFs with novel chemical and physical properties is rarely explored.

Surface plasmon resonance (SPR) is a property in which conduction electrons in the metal nanostructures absorb the incident electromagnetic wave or collectively oscillate and scatter.[10] SPR can couple to the electromagnetic fields emitted by atoms, molecules, or quantum dots located in the vicinity of
the metal nanostructures, leading to a strong modification of the nonradiative and radiative properties of the emitter.\cite{11} Gold nanostructures have been proved to possess efficient photothermal effect due to their SPR, which are able to use for PTT, photothermal controlled drug release, and imaging agent. In addition, gold nanostructures also exhibit antimicrobial activity through precise control of their size and can be used for cancer radiotherapy due to their radiosensitizing effects.\cite{12} Thus, combination of the tunable nature of MOFs with the unique physicochemical properties of gold nanostructures offers an opportunity to develop multifunctional nanocomposites. The key for the combination is to integrate MOFs and gold nanostructures into core–shell structures, where the MOFs shell is grown on the surface of gold nanostructures. Recently, the growth of MOFs onto the surface of gold nanostructures was proposed by using atomic layer deposition (ALD), in which a metal oxide layer was deposited onto the surface of gold nanostructures to grow the MOFs.\cite{13} However, using ALD to construct the core–shell composites often went through a complicated process and caused the poor crystallinity and porosity of MOFs shell. In addition, most of these composites are a dispersion of several metal nanostructures within large MOFs crystals.\cite{14}

Here, we report on a simple, but effective method by using functionalized gold nanorods as seed crystal for the growth of MOFs to construct porphyrinic MOFs-coated gold nanorods (AuNR@MOFs) as a multifunctional theranostic platform (Scheme 1). The chosen porphyrinic MOF is Zr_{6}(TCPP)_{1.5} (TCPP = tetrakis (4-carboxyphenyl)porphyrin), which composes of 6-connected Zr_{6} cluster (Zr_{6}O_{4}(OH)_{4}(H_{2}O)_{6}(OH)_{6}(COO)_{6}) and tetrapod linker (TCPP).\cite{15} Porphyrinic MOFs are promising functional materials for cancer therapy, imaging, and luminescence due to the unique biological and chemical properties offered by the porphyrin building blocks and can be used
to deliver drugs and stabilize metal nanostructures on account of well-defined pore structures and extraordinary high surface areas.\cite{16} The thickness of MOFs shell on the surfaces of AuNR can be controlled by tuning the growth time of MOFs. The camptothecin (CPT) is utilized as a model drug. The large surface area of porphyrinc MOFs can guarantee high drug loading capacity. The photodynamic therapy ability as well as the fluorescence emission nature of the porphyrinic MOFs endows the theranostic function of AuNR@MOFs. These properties make the nanoplatform of AuNR@MOFs not only possess improved drug loading efficiency, near-infrared (NIR) light-induced drug release, and fluorescence imaging, but also produce reactive species with photothermal activity to achieve combined cancer therapy. As expected, AuNR@MOFs@CPT show dramatically enhanced anticancer performance in vitro and in vivo studies.

2. Results and Discussion

2.1. Preparation and Characterization of AuNR@MOFs

In this study, AuNRs were prepared according to a seed-mediated growth method.\cite{17} Transmission electron microscopy (TEM) images of AuNRs show that the aspect ratio is approximate 3.7 (Figure 1A). The surfaces of AuNRs were functionalized with lipoic acid and poly(ethylene glycol) (PEG)-SH polymer. The well-dispersed surface carboxylated AuNRs with high physical–chemical stability can use as the cores for heterogeneous nucleation for the growth of MOFs due to the coordination interactions between carboxyl groups of AuNRs and zirconium nodes (Figure 1B). AuNR@MOFs was fabricated by the solvothermal method using functionalized AuNRs as crystal nucleus (Figures S1 and S2, Supporting Information). As shown in Figure 1C, the mean length and width of AuNR@MOFs are approximate 53.8 ± 2.1 and 25.2 ± 1.7 nm. Localized MOFs nucleation growth on the surfaces of AuNRs leads to the formation of the core–shell composites. To confirm the components of AuNR@MOFs, the composites were characterized by transmission electron microscope/energy-dispersive X-ray spectrometry (TEM–EDS). The characteristic peaks corresponding to the zirconium and gold elements prove the formation of the composites (Figure 1D). Powder X-ray diffraction (PXRD) pattern of AuNR@MOFs demonstrate a good match for the diffraction peaks between the porphyrin MOFs phase of AuNR@MOFs and pure porphyrin MOFs and exhibited two additional strong diffraction peaks at 38.1 and 44.4° due to the peaks of the AuNRs.
at (111) and (200) crystal plane (Figure 1E). These results reveal the formation of MOFs phase onto the surfaces of AuNRs. Furthermore, the thickness of MOFs shell on the surfaces of AuNRs can be controlled by the MOFs growth time. The TEM images of AuNR@MOFs show that the thickness of MOF shell is about 8.1 ± 2.3 nm after the MOF-formation reaction time at 4 h and increasing the reaction time to 6 h leads to a thickness of 13.2 ± 1.6 nm (Figure 1F,G).

To further demonstrate the formation of MOFs shell, a typical core−shell composite of AuNR@MOFs was measured by TEM−EDS elemental mapping. The signals corresponding to the gold, zirconium, and carbon can be detected (Figure 1H). Moreover, we found that the shell thickness of AuNR@MOFs increased with the growth time of MOFs. The porosity of AuNR@MOFs was evaluated by nitrogen adsorption−desorption studies at 77 K. The maximum N2 uptake is up to 210 cm3 g−1 standard temperature and pressure (STP) and around 1.6 ± 0.3 nm pore size distribution is obtained, indicating AuNR@MOFs possess high surface area and porosity (Figure S3, Supporting Information). The optical properties of AuNR series were investigated by UV−vis and fluorescence spectrum. For AuNR@MOFs, the longitudinal band remained in the NIR region and the additional peaks from porphyrin absorbance appeared, demonstrating the formation of core−shell composites (Figure 2A).

The fluorescence spectrum of AuNR@MOFs exhibits the strong fluorescence emission peak at 650 nm under the excitation of 420 nm due to the presence of porphyrinic MOFs shell (Figure 2B). These results provide an opportunity for AuNR@MOFs to be used as a general theranostic platform.

2.2. Loading and Photothermal-Trigger Release of Camptothecin

Given the high surface area and the porosity of porphyrinic MOFs, we supposed AuNR@MOFs could be used as a superior drug delivery system. As AuNR@MOFs with nanopore was...
positive charge in water, small molecule drugs had the possibility to be encapsulated into the nanoporous spaces through π-π stacking and electrostatic interaction.[18] The loading and release behaviors of AuNR@MOFs were investigated by using CPT, a chemotherapy drug in clinic, as the model drug.[19] We demonstrated that the loading capacity of CPT was more than 25% in weight through the thermogravimetric analysis (TGA) and UV–vis spectroscopy (Figures S4 and S5, Supporting Information). Moreover, the surface potential of AuNR@MOFs reduced from 18.8 to 5.4 mV after loaded CPT because of the negatively charged CPT molecules immobilized onto the positively charged AuNR@MOFs (Figure 2C). We also further explored the fluorescence spectrums of free CPT, AuNR@MOFs and AuNR@MOFs@CPT (Figure S6A–C, Supporting Information). The fluorescence emission of AuNR@MOFs@CPT presents a significantly enhanced peak between 430 and 460 nm compare with AuNR@MOFs due to the presence of CPT (Figure S6D, Supporting Information). Interestingly, the fluorescence emission range of free CPT is inconsistent with the CPT molecule in AuNR@MOFs@CPT. This might be the result of the fluorescence resonance energy transfer from CPT molecules to the porphyrin building blocks due to the spectral overlap between the CPT molecules emission and the porphyrin building blocks absorption (Figure S6D, Supporting Information). Additionally, the ability of AuNR@MOFs in buffer (10 × 10^-3 M HEPES, pH 7.4) was investigated. The hydrodynamic diameter and polydispersity index (PDI) of AuNR@MOFs in buffer show a little change during 48 h, confirming that AuNR@MOFs is relatively stable at normal physiological conditions (Figure S7A-C, Supporting Information). PXRD data also indicates that AuNR@MOFs can retain their crystallinity in buffer for 48 h (Figure S7D, Supporting Information). Moreover, the size distributions of AuNR@MOFs@CPT in water and dulbecco’s modified eagle medium (DMEM) were evaluated by dynamic light scattering (Figure 2D). Only a small change in hydrodynamic diameter and PDI were observed during 48 h in DMEM, indicating that AuNR@MOFs@CPT is stable under normal physiological conditions (Figure S8, Supporting Information).

To confirm the photothermal ability, the temperature trends of AuNR@MOFs solution were recorded under irradiation with 808 nm laser. For 100 µg mL^-1 AuNR@MOFs dispersion, the system temperature can rise from 28.5 to 51.5 °C upon an irradiation as short as 2 min, and finally as high as ~73.4 °C after 10 min irradiation (Figure S9A, Supporting Information). Such hyperthermia can efficiently kill tumor cells within a short time. By contrast, the temperature of pure water only elevated ~2.3 °C after 10 min under the same test conditions. According to Roper’s method, the photothermal conversion efficiency of AuNR@MOFs was determined to be ~20.6% based on the linear fitting of data points deduced from the cooling curve in Figure S9B in the Supporting Information.[20] Furthermore, the photothermal effect of AuNR@MOFs@CPT was investigated by monitoring the temperature elevation of AuNR@MOFs@CPT aqueous dispersions at various concentrations (0, 50, 100, 150 µg mL^-1) under 808 nm laser irradiation for 14 min. When the concentration of AuNR@MOFs@CPT gradually increased from 0 to 150 µg mL^-1, the corresponding temperature elevation (ΔT) after 2 min irradiation was ~0.7, 24.5, 33.3, and 39.1 °C, respectively (Figure 2F). Additionally, the temperature rising rate of AuNR@MOFs@CPT solution increases rapidly with laser power (Figure 2F). The results suggest that the photothermal effect of AuNR@MOFs@CPT could be conveniently obtained and finely regulated by controlling the laser power as well as the concentration of AuNR@MOFs@CPT. To simulate the physiological environment and tumor microenvironment, the release of CPT from AuNR@MOFs@CPT was investigated at pH 5.0 and 7.4. The photothermal controlled CPT release was performed by tuning 808 nm laser irradiation to maintain a hyperthermia environment at 50 ± 2.0 °C. Due to the poor solubility of CPT at room temperature, less than 10% CPT was released after 24 h incubation at pH 5.0 and 7.4 in dark. In contrast, the release rates of CPT at pH 5.0 and 7.4 significantly increased with 808 nm laser irradiation. It can be supposed that the photothermal effect would further enhance the molecular motion of CPT within AuNR@MOFs and therefore, enable the release of loaded CPT (Figure S10, Supporting Information).

2.3. Photoinduced Reactive Oxygen Species (ROS) Generation and NIR Light-Trigger CPT Intracellular Release

To evaluate the photodynamic activity, 2',7'-dichlorodihydrofluorescein (DCFH) was used to detect the amount of O₂ by monitoring the increased fluorescence emission of DCFH at 523 nm. The fluorescence changes of DCFH in the control group were not obvious under 660 nm light-emitting diode (LED) irradiation. In contrast, the fluorescence of DCFH rapidly increased for AuNR@MOFs@CPT with 660 nm LED irradiation due to the continuous production of reactive oxidase species (Figure S11, Supporting Information). We further investigated the O₂ production ability of AuNRs, porphyrinic MOFs, and AuNR@MOFs under 660 nm LED irradiation by monitoring the absorption of anthracene-9,10-dipropionic acid (ADPA) at 378 nm over time (Figure S12A-C, Supporting Information). The results indicate that AuNR@MOFs presents a relatively enhanced ability to generate O₂ compared with porphyrinic MOFs under light irradiation due to the existence of gold nanorods (Figure S12D, Supporting Information). This is mainly attributed to the enhanced light absorption and strong electromagnetic field effect of gold nanorods surface. Firstly, gold nanorods can strongly scatter and absorb light, especially resonant with their surface plasmons at wavelengths. Additionally, the longitudinal localized surface plasmon resonance (LSPR) of gold nanorods enhances the electromagnetic field that is produced at the metal surface due to photon confinement, and so controls both ROS production and luminescence via multiphoton excitation of electrons in water and gold followed by hot plasma generation in water.[21]

Furthermore, the intracellular ROS generation of AuNR@MOFs@CPT was investigated with DCFH as the ROS probe, which emitted green fluorescence in the presence of ROS by confocal laser scanning microscopy (CLSM) observation. The 4T1 cells cultured with AuNR@MOFs@CPT under the irradiation of 660 nm LED exhibited significant green fluorescence by CLSM observation (Figure 3A). Comparatively, when the 4T1 cells were cultured with AuNR@MOFs@CPT in dark, the green fluorescence was negligible (Figure 3B). Thus, AuNR@MOFs@CPT can produce ROS in the cells under the irradiation...
Figure 3. The in vitro antitumor performance. A) The intracellular ROS generation of AuNR@MOFs@CPT in dark and B) with 660 nm LED irradiation. A1-B1: green fluorescence of DCFH-DA. A2-B2: bright field. A3-B3: merge image. C) The cytotoxicity of AuNR@MOFs. D) The cytotoxicity of AuNR@MOFs@CPT. E) Flow cytometry analysis of the dark and light toxicities of AuNR@MOFs@CPT without any treatment as the blank control (Q1: dead cells; Q2: late apoptosis or necrosis cells; Q3: early apoptosis cells; Q4: vital cells).
with 660 nm LED. The intracellular drug release behavior of AuNR@MOFs@CPT was measured with 4T1 cells (Figure S13, Supporting Information). We found that the 4T1 cells treated with AuNR@MOFs@CPT in dark exhibited weak blue fluorescence signal. However, the intracellular blue fluorescence intensity obviously enhanced under the irradiation with 808 nm laser. These results indicated that the intracellular release of CPT could be accelerated by the photothermal effect of AuNRs.

2.4. In Vitro Cytotoxicity Assay

Given the superior performance of the AuNR@MOFs@CPT, cellular uptake and in vitro therapeutic effects were investigated. The murine breast tumor 4T1 cells were incubated with AuNR@MOFs@CPT for 4, 6, or 8 h. The red fluorescent signal from the endocytosis of the AuNR@MOFs@CPT gradually increased under CLSM observation (Figure S14, Supporting Information). The optimized cellular uptake time was 6 h. To investigate the chemotherapy effect of the CPT, the methyl thiazolyl tetrazolium (MTT) assay of free CPT was performed, indicating the lower concentration of CPT can remarkably affect the cell viability when the cells were incubated with AuNR@MOFs@CPT under 660 nm LED irradiation, 53% of the cell destruction was presented. Compared to the cells treated with AuNR@MOFs@CPT alone or AuNR@MOFs@CPT without light irradiation, the cell survival ratio was approximate 90% (Figure 3D). This result might be induced by the leakage of CPT from AuNR@MOFs@CPT. When the cells were incubated with AuNR@MOFs@CPT under 660 nm LED irradiation, 37% of the cells were killed, compared with 32% of AuNR@MOFs@CPT with the sizes of 50–100 nm are appropriate for rapid accumulation and efficient tumor tissues accumulation and photothermal effect in tumor position.[22] Furthermore, AuNR@MOFs@CPT with the sizes of 50–100 nm are appropriate for prolonged circulation and efficient tumor tissues accumulation by the EPR effect. In addition, the rod-like nanostructures can accumulate at higher levels and penetrate tumor tissues more rapidly than size-matched spheres.[23] Meanwhile, we also found that the fluorescence in liver was strong due to the mononuclear phagocytic system (MPS) in vivo. To quantitatively assess the biodistribution of AuNR@MOFs@CPT, the amounts of Au in major organs (heart, liver, spleen, lung, kidney) and the tumor tissues were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES). As showed in Figure 5E, Au element mainly accumulated in tumor tissue and liver. To demonstrate the photothermal activity of AuNR@MOFs@CPT inside the tumor, in vivo photothermal imaging was evaluated after 24 h intravenous injection of AuNR@MOFs@CPT to 4T1 tumor-bearing mice. We found that the fluorescence signal increased continuously in the tumor region and came to a head at 24 h post-injection (Figure 4A). Thus, 24 h was chosen as the optimal time point of light irradiation for subsequent PDT and/or PTT (Figure 4C). After 24 h, the mice were sacrificed, and the tumor and main organs were harvested for imaging (Figure 4B,D). AuNR@MOFs@CPT can effectively accumulate and retain in tumor tissues via the enhanced permeability and retention (EPR) effect. Firstly, AuNR@MOFs@CPT with good hydrophilicity and stability at physiological conditions has resulted in systems with prolonged circulation that are able to evade RES recognition and elimination and therefore exploit the EPR effect by multiple channels through the tumor sites.[22] Furthermore, AuNR@MOFs@CPT with the sizes of 50–100 nm are appropriate for prolonged circulation and efficient tumor tissues accumulation by the EPR effect.[23] In addition, the rod-like nanostructures can accumulate at higher levels and penetrate tumor tissues more rapidly than size-matched spheres.[23] Meanwhile, we also found that the fluorescence in liver was strong due to the mononuclear phagocytic system (MPS) in vivo. To quantitatively assess the biodistribution of AuNR@MOFs@CPT, the amounts of Au in major organs (heart, liver, spleen, lung, kidney) and the tumor tissues were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES). As showed in Figure 5E, Au element mainly accumulated in tumor tissue and liver. To demonstrate the photothermal activity of AuNR@MOFs@CPT inside the tumor, in vivo photothermal imaging was evaluated after 24 h intravenous injection of AuNR@MOFs@CPT and monitored by a near-infrared thermal camera (Figure 4F,G). For AuNR@MOFs@CPT group, the temperature of the tumor increased rapidly from 28.5 to 48.4 °C after irradiated for 2 min, and then came to a plateau temperature of 54.8 ± 1.2 °C, which was high enough to kill tumor cells. In contrast, the temperature of the tumor had no significant change in the control group. These results indicated that AuNR@MOFs@CPT possessed favorable accumulation and photothermal effect in tumor position.

2.6. In Vivo Antitumor Performance of AuNR@MOFs@CPT

Encouraged by the excellent results in vitro and the tumor accumulation of AuNR@MOFs@CPT, we further performed the combined therapy on mice bearing 4T1 tumors in vivo. The mice were randomly divided into ten groups (6 mice per group). The mice were treated with free CPT, AuNR@MOFs or AuNR@MOFs@CPT solution by intravenous method and then the laser irradiation was implemented after 24 h at 1th and 9th d. The tumor volumes were recorded by a caliper for
For systematic comparison, the tumors of the control group increased rapidly as time went on. The combination of PDT and chemotherapy using AuNR@MOFs or AuNR@MOFs@CPT with 660 nm laser irradiation could partially inhibit the tumor growth. While the combination of the chemotherapy of photothermal-induced CPT release and PTT using AuNR@MOFs or AuNR@MOFs@CPT with 808 nm laser irradiation showed the improved growth inhibition effect compared to the combination of PDT and chemotherapy. Importantly, the tumor growth was remarkably suppressed under the treatment of AuNR@MOFs@CPT with dual laser irradiation compared to the other groups. After 18 d post-treatment, all mice were sacrificed. The digital photos of excised tumors from typical mice visually exhibited that the mice treated with AuNR@MOFs@CPT under dual laser irradiation obtained smaller tumor sizes than the other control groups (Figure 5C,D). Moreover, the body weight of all mice in the ten groups had no significant change, indicating negligible systemic toxicity of AuNR@MOFs or AuNR@MOFs@CPT and the optimal antitumor efficacy of AuNR@MOFs@CPT (Figure 5B).

To further demonstrate the antitumor performance of AuNR@MOFs@CPT at the cellular level, histological analysis of the tumor tissues from typical mice was carried out. Hematoxylin and eosin (H&E) staining clearly displayed that most of tumor tissue cells became apoptotic and necrotic and were killed after the mice treated with AuNR@MOFs@CPT under 660 and 808 nm laser irradiation. In contrast, tumor cells of

Figure 4. In vivo biodistribution and photothermal conversion studies. A) The in vivo fluorescence images of the mice after intravenous injection of AuNR@MOFs@CPT and B) Ex vivo imaging of tumor and major organs after 24 h post-injection. C) Quantitative fluorescence curve of tumor tissues. D) Quantitative fluorescence intensity determination of major organs and tumor tissue. E) The quantitative analysis on gold element by ICP-AES in main organs and tumor tissue after 24 h post-injection. F) The in vivo thermal images of the mice after intravenous injection of PBS and AuNR@MOFs@CPT with 808 nm laser irradiation. G) Temperature change curve of tumor tissues as a function of irradiation time.
the other groups partially or largely retained their normal cell morphology (Figure 5E). Moreover, in situ terminal deoxynucleotidyl transerferase dUTP nick end labeling (TUNEL) staining of the tumor tissues also displayed that obvious destruction after the mice treated with AuNR@MOFs@CPT under 660 and 808 nm laser irradiation, in line with the result of H&E (Figure 5F). These results indicated that AuNR@MOFs@CPT exhibited favorable antitumor performance. The pharmacodynamic study demonstrated that AuNR@MOFs@CPT possessed relatively long circulation time in the blood (Figure S17, Supporting Information). Furthermore, the histological analysis of the major organs (spleen, heart, liver, kidney, and lung) of each group was performed to evaluate the biosafety of AuNR@MOFs@CPT in vivo, indicating no obvious damage to normal tissues, such as inflammatory response and necrosis (Figure S18, Supporting Information). It turned out that the nanomedicine of AuNR@MOFs@CPT possessed the favorable biocompatibility for combination therapy in vivo. Additionally,
AuNR@MOFs@CPT also dramatically increased the survival rate of tumor-bearing mice (Figure 6A). Interestingly, since 4T1 cancer cells possessed highly metastatic, we found the obvious hepatic metastases of control group by the histological analysis of liver compared to the group treated with AuNR@MOFs@CPT (Figure 6B,C). That's probably because the accumulation of AuNR@MOFs@CPT in liver and tumor position can inhibit hepatic metastases. The digital photos of the mice treated with AuNR@MOFs@CPT for 50 d visually showed that the tumors were almost entirely disappeared (Figure 6D).

The reason for the dramatic synergistic antitumor performance in vitro and in vivo studies probably thought to the following aspects. First of all, PDT relied on the fact that local light excitation of AuNR@MOFs@CPT could generate high ROS that can induce lipid peroxidation and improve the membrane permeability, for irreversibly damaging of tumor cells.[26] What's more, the photothermal effect of AuNR@MOFs@CPT can produce the local hyperthermia under NIR light radiation, which was able to kill the tumor cells and not depended on the hypoxic tumor environment. Meanwhile, the local hyperthermia has been demonstrated to promote the blood flow so as to enhance oxygen supply, and eventually improve the synergistic effect with PDT.[27] Moreover, the local hyperthermia can increase the permeability of cell membrane and accelerate the CPT release from AuNR@MOFs@CPT, leading to the rapid intratumoral CPT release and improving the cytotoxicity of chemotherapy. Additionally, the chemotherapy of photothermal-trigger CPT release and PDT as well as PTT can supplement mutually, improve the antitumor performance and reduce the risk of tumor recurrence.

3. Conclusion

In summary, we have developed an effective method to construct porphyrinic MOFs-coated gold nanorods by using functionalized gold nanorods as seed crystal for the growth of MOFs. In this system, AuNRs are individually incorporated within porphyrinic MOFs to form delicate core–shell composites, which possess the improved drug loading efficiency, NIR light-trigger drug release and fluorescence imaging. We further demonstrated that AuNR@MOFs@CPT could be used as a multifunctional theranostic nanoplatform for tumor photodynamic/thermal/chemo-combined therapy and show distinctively synergistic efficiency for damaging the tumor cells in vitro and in vivo. In addition, it was also found that this nanomedicine platform has the ability of suppressing the tumor metastasis in vivo. We believe this core–shell theranostic platform will find great potential in tumor treatment.

4. Experimental Section

Preparation of AuNRs: For the seed solution, CTAB solution (1.0 mL, 0.20 M) was mixed with HAuCl₄ (1.0 mL, 0.5 × 10⁻³ M) and then ice-cold NaBH₄ (0.12 mL, 0.01 M) was added. After the seed solution was vigorous stirred for 2 min, it was kept at 25 °C for 2 h and resulted in the formation of a brownish-yellow solution. The growth solution was prepared by mixing together CTAB (0.1 M, 200 mL), AgNO₃ (4 × 10⁻¹ M, 5.6 mL) and HAuCl₄ (23 × 10⁻¹ M, 6.5 mL) in 250 mL flask. Ascorbic acid (0.08 M, 1.8 mL) was dropwise added to the mixture,
until the solution became colorless. Finally, 1.8 mL of the seed solution was added to the mixture at 30 °C. The color of the mixture gradually changed within 10–15 min. The mixture of the AuNRs growth was kept at 27–30 °C for 12 h.

Preparation of AuNR@MOFs: Functionalized AuNRs were washed twice with DMF and then mixed with TCPP (10 mg, 0.013 mmol), benzoic acid (0.28 g, 2.3 mmol), and ZrOCl2·8H2O (30 mg, 0.093 mmol). The mixture was stirred at room temperature for 1 h and then was heated to 90 °C for 4 h. After cooling down, the core–shell composites were collected via centrifugation (11000 rpm, 20 min) followed by washing with DMF, 1% triethylamine in ethanol (v/v), and ethanol successively for 3 times.

Intracellular Photothermal-Trigger CPT Release: The CPT loading represent the amount of CPT in the composites (g mL⁻¹ × 10⁻³). Where M_Loading represents the amount of CPT in the composites and M_Total is the total amount of CPT-loading composites. M_Loading was calculated by the following equation: M_Loading = M_Total – M_Nonloading. Where M_Total is the total amount of initially fed for loading and M_Nonloading is the amount of CPT remaining in the supernatant after centrifuging the sample. For the long-term drug release in vitro, the CPT loaded composites (1.0 mg) solution was added to the dialysis bags (MW 5000 Da), and then directly immersed into 5.0 mL of HEPES buffer solution with or without 808 nm laser irradiation. At various times, 1.0 mL of the dialysate was collected and the remaining dialysate supplemented with the same amount of fresh buffer solution. The release amount of CPT in buffer solution was determined on the basis of the fluorescence emission intensity at 440 nm.

Photothermal Effect of AuNR@MOFs@CPT: 1.0 mL of AuNR@MOFs@CPT aqueous solution was added into a 2 mL plastic centrifuge tube. The top of plastic centrifuge tube was fixed and a fiber-coupled continuous semiconductor diode laser was used as the light source.

Generation and Detection of ROS: 5 μL of DCFC solution (10 × 10⁻³ μL) was mixed with 1 mL of AuNR@MOFs@CPT (40 μg mL⁻¹) composites solution. The control groups, 5 μL of DCFC solution (10 × 10⁻³ μL) was mixed with 1 mL HEPES buffer solution with or without 808 nm laser irradiation. At various times, 1.0 mL of the dialysate was collected and the remaining dialysate supplemented with the same amount of fresh buffer solution. The release amount of CPT in buffer solution was determined on the basis of the fluorescence emission intensity at 440 nm.

In Vivo Fluorescence and Photothermal Imaging: When the tumors grew to 200–300 mm³ in volume, 100 μL of AuNR@MOFs@CPT solution (containing TCPP: 3.5 mg kg⁻¹) were injected into the mice by intravenous method. Thereafter, the mice were anesthetized and imaged in a small animal imaging system (PE Spectrum & Quantum FX) at 2, 6, 12, 18, 24, 36, and 48 h post-injection. The excitation wavelength was 640 nm and the fluorescence emission at 650 nm was collected. For the tissue distribution study of the composites, the mice were sacrificed at 24 h post-injection, and major organs were collected for ex vivo imaging. To quantitatively assess the biodistribution of the AuNR@MOFs@CPT, major organs (heart, liver, spleen, lung, and kidney) and the tumor tissues were obtained and digested to determine the amounts of Au through using ICP-AES measures at 24 h post-injection. When the tumors reached an approximate size of 200 mm³ in volume, 100 μL of AuNR@MOFs@CPT solution (containing TCPP: 3.5 mg kg⁻¹) were injected into the mice by intravenous method. The mice of the control group were injected with PBS buffer. Infrared thermal imaging was measured by a camera (FLIR A 5) under irradiation with an 808 nm laser at a power density of 1.5 W cm⁻² and the temperature of mice was recorded by BM_IR software.

In Vivo Antitumor Study and Histochimistry Analysis: Animals and Tumor Model: the female BALB/c mice (5-week-old) were bought from Wuhan University Animal Biosafety Level III Lab and used for animal experiments. All animal experiments were agreed with institutional
animal use and care regulations from Wuhan University. The tumor-bearing mice were obtained by injecting 4T1 cells (1 × 10⁶ cells) into subcutaneous of female mice on the right back of hind leg region. All animal experiments were carried out according to the guidelines of laboratory animals, which were defined by the Wuhan University Center for Animal Experiment/A3-Lab. When the tumors reached an approximate size of 200 mm³ in volume, the mice were randomly divided into 10 groups (6 mice per group). Then, tumor-bearing mice were treated with (1) PBS, (2) AuNR@MOFs, (3) AuNR@MOFs@CPT, (4) Free CPT (containing CPT: 20 µg), (5) AuNR@MOFs (containing TCPP: 3.5 mg kg⁻¹) with laser (660 nm: 4 min, 0.22 W cm⁻²) irradiation, (6) AuNR@MOFs (containing TCPP: 3.5 mg kg⁻¹) with laser (808 nm: 2 min, 1.5 W cm⁻²) irradiation, (7) AuNR@MOFs (containing TCPP: 3.5 mg kg⁻¹) with laser (660 nm: 4 min, 0.22 W cm⁻² and 808 nm: 2 min, 1.5 W cm⁻²) irradiation, (8) AuNR@MOFs@CPT (containing TCPP: 3.5 mg kg⁻¹) with laser (808 nm: 2 min, 1.5 W cm⁻²) irradiation, (9) AuNR@MOFs@CPT (containing TCPP: 3.5 mg kg⁻¹) with laser (660 nm: 4 min, 0.22 W cm⁻²) irradiation and (10) AuNR@MOFs@CPT (containing TCPP: 3.5 mg kg⁻¹) with laser (660 nm: 4 min, 0.22 W cm⁻² and 808 nm: 2 min, 1.5 W cm⁻²) irradiation. The mice were performed with a dose of 100 µL and then the laser irradiation was implemented after 24 h at 1th and 9th d. Tumor size and mice weight were measured every day. Tumor size was recorded by a caliper and tumor volume was defined as: \[ V = \frac{W^2 	imes L}{2}, \] where \( W \) and \( L \) were the shortest and longest diameters of tumors, respectively. Relative tumor volume was calculated using the formula: \[ \frac{V_0}{V_t} = \frac{W_t}{W_0} \times \frac{L_t}{L_0}, \] where \( V_0 \) was the tumor volume when the injection was initiated). On day 18, all mice were killed and then the tumors were excised and weighed. Simultaneously, the main organs (heart, liver, spleen, lung, and kidney) were also obtained and utilized for histology analysis. For pharmacokinetic studies, blood samples were taken at the 1, 2, 4, 6, 8, 10, 12, 16, and 24 h time points after post-treatment. Appropriate DMSO was added and then the samples were treated under ultrasound for 5 min. The samples were centrifuged at 2000 r/min for 3 min and the supernate was measured by fluorescence spectroscopy (Ex = 420 and Em = 650 nm).

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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