Fluorescent Imaging-Guided Chemotherapy-and-Photodynamic Dual Therapy with Nanoscale Porphyrin Metal–Organic Framework

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1. Introduction

Imaging-guided therapy systems (IGTSs) are revolutionary techniques used in cancer treatment due to their safety and efficiency. IGTSs should have tunable compositions for bioimaging, a suitable size and shape for biotransfer, sufficient channels and/or pores for drug loading, and intrinsic biocompatibility. Here, a biocompatible nanoscale zirconium-porphyrin metal–organic framework (NPMOF)-based IGTS that is prepared using a microemulsion strategy and carefully tuned reaction conditions is reported. A high content of porphyrin (59.8%) allows the achievement of efficient fluorescent imaging and photodynamic therapy (PDT). The 1D channel of the Kagome topology of NPMOFs provides a 109% doxorubicin loading and pH-response smart release for chemotherapy. The fluorescence guiding of the chemotherapy-and-PDT dual system is confirmed by the concentration of NPMOFs at cancer sites after irradiation with a laser and doxorubicin release, while low toxicity is observed in normal tissues. NPMOFs are established as a promising platform for the early diagnosis of cancer and initial therapy.

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1. Introduction

Traditional methods (i.e., surgical resection, chemotherapy, and radiotherapy) still dominate cancer treatment but result in side effects and high recurrence rates.[1,2] Multifunctional therapeutic platforms that combine new treatment modes, such as photothermal therapy (PTT) and photodynamic therapy (PDT), have all the advantages of a single therapy in improving efficiency with the added benefits of minimizing side effects, reducing drug dosage, and overcoming drug resistance.[3–5] Imaging-guided therapy systems (IGTSs) further integrate visualization technology to exert the combination of imaging and multitherapy.[6–9] The distribution and evolution of the tumor/drug are expected to enhance both the efficiency and safety of the treatment through time- and position-resolved modes.[4,10–12] PDT generates reactive oxygen species, such as singlet oxygen, with photosensitizers and oxygen with light irradiation, resulting in oxidative damage to the tumor tissue.[13,14]
PDT is a safe method due to its invasiveness and ablation of cancer cells in a time and site-controllable manner.\textsuperscript{[15,16]} Patients can tolerate repeated doses of PDT, and therefore it has a potential for long-term service without producing drug resistance.\textsuperscript{[17]} Porphyrin-based PDT has been adopted for cancer treatment.\textsuperscript{[18,19]}

Chemotherapy is greatly improved with nanoengineering.\textsuperscript{[9,20–24]} Many hydrophobic anticancer drugs have low bioavailability and unfavorable pharmacokinetics. Smart drug delivery provides both high drug loading and decreased biotoxicity. Metal–organic frameworks (MOFs) are a class of porous materials with defined structures, tunable porosity, and robust stability, so MOFs have versatile applications in drug encapsulation and delivery.\textsuperscript{[23,25,26]}

The combination of chemotherapy with PDT has resulted in efficient therapy and negligible toxicity.\textsuperscript{[7,27]} Molecular probes, biodegradable polymer, and aerosol OT (AOT)-alginate nanoparticles were used as carriers to deliver drugs and photosensitizers.\textsuperscript{[28–30]} However, in vivo imaging-guided therapy was essential for improving the efficiency and safety of the treatment. A highly porous and stable porphyrin MOF (PCN-222 or MOF-545) was previously reported as catalyst in peroxidase-like oxidation reaction, but the strong fluorescence of the MOF was not fully developed.\textsuperscript{[31,32]} Herein, we integrated the properties of a porphyrin and MOFs to build an IGTS for cancer probing and treatment. Nanoscale porphyrin MOFs (NPMOFs) were built with biocompatible Zr ions and meso-tetrakis(4-carboxyl)-21H,23H-porphine (TCPP) by a microemulsion method. The one-pot fabrication procedure was much simpler and more robust than previously reported methods for IGTSs synthesis, such as micelle polymers and the lanthanide upconversion nanoparticles.\textsuperscript{[8,24]} The large channel of NPMOFs could load doxorubicin (DOX) as high as 109%. The high content of porphyrin (59.8%) provided a strong fluorescence and rapid singlet oxygen generation. Porphyrins are seldom used as fluorescent imaging agents directly because of their hydrophobic nature and tendency to aggregate.\textsuperscript{[15,31]} but NPMOFs retain the photophysical properties of the porphyrin. The red fluorescence within the bioimaging window showed a high penetration depth for cancer imaging and drug tracking. Smart DOX release and selective PDT killed cancer cells effectively. Red fluorescence guiding of the dual therapy was confirmed in cancer cells, zebrafish, and mice models. The tumor-bearing mouse experiment demonstrated quick localization of NPMOFs at cancer sites and efficient therapy after laser irradiation and DOX release. The circulation and clearance of NPMOFs validated the safety of the NPMOF probe. This is the first porphyrin MOF reported as a biocompatible fluorescent IGTS to the best of our knowledge.

2. Results and Discussion

Nanosize is the first requirement of an IGTS probe, although bulk needle-shaped MOF-545 and PCN-222 have been reported only with Zr ions and TCPP as the precursors.\textsuperscript{[31,32]} Therefore, we attempted to obtain nanoscale POMOFs (NPMOFs) using a microemulsion template and the auxiliary ligand method.\textsuperscript{[33]} Benzoic acid (BA), PEG-6000, and cetyltrimethylammonium bromide (CTAB) were selected as the auxiliary ligand, capping agent, and surfactant, respectively, to regulate the PMOF size and hydrophilicity.\textsuperscript{[34]} Different morphologies were clearly observed in PMOFs with different BA/CTAB ratios (Figure S1a–c, Supporting Information). Hydrophilic and biocompatible PEG-6000 improved the dispersion of NPMOFs. NPMOFs dispersed well in water to form a clear and transparent solution that was different from the TCPP and bulk PMOFs (Figure S1d, Supporting Information). The precursor ratio was also critical for the size of the PMOFs as follows: smaller PMOFs were obtained by diluting the solution (Figure S1e,f, Supporting Information). The time-dependent growth process of NPMOFs was recorded to investigate the formation mechanism (Figure 1 and Figure S2, Supporting Information). Crystal seeds (6 nm) formed in 10 h (Figure S2a, Supporting Information). Spherical structures (143 nm) were observed to form via rapid growth in 3D until 16 h (Figure 1a,d, and Figure S2b, Supporting Information). Then, the crystallization of the NPMOFs along the channel became much faster than that in the other directions, resulting in spindle-like particles within 24 h (Figure 1b,e, and Figure S2c, Supporting Information) and rod-like PMOFs from 40 h (Figure 1c,f, and Figure S2e, Supporting Information) to 48 h (Figure S2f, Supporting Information). However, the same channel size of 1.75 nm was observed in all NPMOFs, even with different reaction times. The 1D channel of NPMOFs was further illustrated.
by a simulated crystal structure (Figure 1g) and the distance between the Zr clusters observed in high-resolution transmission electron microscopy (TEM) images (Figure 1h). Zr clusters (gray polyhedron in Figure 1g) were linked through the planar TCPP to form a Kagome-type topology.

Spindle-like NPMOFs (155 × 260 nm) were selected as the final IGTS platform for drug loading and PDT efficiency (Figure 1b). The size of the NPMOFs was suitable for PDT because the diffusion length of singlet oxygen (1O2) is in the range of 90–120 nm in aqueous environment and 20–220 nm inside cells. The spindle-like structure and 1D channel of NPMOFs provided high DOX-loading efficiency and fast adsorption–desorption kinetics. The zeta potential of the PEG-coated NPMOFs was −3.4 mV, whereas that of the uncoated NPMOFs was −22.1 mV. The changes in water dispersion and zeta potential of NPMOFs confirmed the surface coating by PEG (Figure S3, Supporting Information).

The structure of the NPMOFs was further confirmed by the powder X-ray diffraction (PXRD) patterns of the NPMOFs, bulk PMOFs, and simulated data (Figure S4, Supporting Information). Concisely matched peaks in small angle sections validated that the crystal structure of NPMOFs was maintained. The broad full width at half-maximum indicated the nanosize of the NPMOFs, which was calculated based on the Scherrer equation. The peak emerging at 15°–35° was attributed to the scattering effect of the nanoparticles. Nitrogen adsorption indicated that the Brunauer–Emmett–Teller (BET) surface area of the NPMOFs was 419 m^2\cdot g^{-1} (Figure S5a, Supporting Information). Two types of DFT pores with sizes of 1.75 and 3.5 nm were observed (Figure S5b, Supporting Information), which was consistent with the results from the TEM imaging and theoretical predictions (Figure 1g,h). Fourier transform infrared spectra of the NPMOFs and TCPP validated that TCPP was successfully integrated into the NPMOFs (Figure S6, Supporting Information).

The composition of the NPMOFs was investigated using a thermogravimetric analysis (TGA, Figure S7, Supporting Information) and inductively coupled plasma-mass spectrometry (ICP-MS). The complete decomposition of the NPMOFs at 425.6 °C indicated their high thermal stability. In total, 59.8% TCPP and 21.2% Zr were observed in NPMOFs; thus, Zr ions did not coordinate with the nitrogen atoms in the porphyrin center. The high content of TCPP illustrated the rapid DOX loading property. The loading efficiency was 44.3% after 8 h and 58.5% after 72 h, which ensured a high biocompatibility and low side effects to normal tissues. However, in cancerous conditions (pH 7.0, e.g., lysosome), a faster release was observed (34.3% after 8 h and 58.5% after 72 h) indicating a capacity for high therapeutic effects (Figure 2e).

The PDT ability of the NPMOFs was investigated in vitro using the 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA) method. The consumed ABDA (recorded with a 380 nm UV–vis absorbance) represented the capacity of singlet oxygen generation (Figure 2c,d). In contrast, the ABDA absorbance showed high tolerance to single NPMOFs and light (Figure S8, Supporting Information). The 1O2 generation rate can be calculated with the equation:

\[
\frac{\text{rate}}{\text{time}} = \frac{A}{t} = c \cdot e^{\left(-\frac{\pi \lambda}{\lambda} \cdot t\right)} + A_1
\]

where \( A \) is the absorbance of ABDA at 380 nm, and \( t \) is the irradiation time, \( A_1 \) and \( c \) are fitting parameters. The 1O2 generation rate (\( \nu \)) was 0.0725 min\(^{-1}\) for NPMOFs. The singlet oxygen quantum yield \( (Y) \) of the NPMOFs was calculated as 47.4%, which indicates their high PDT efficiency. We selected 655 nm laser irradiation for 1O2 generation from the NPMOFs with a high penetration depth.

DOX, a popular anticancer drug, was selected as a model to investigate its adsorption by NPMOFs and pH-responsive release behavior. Figure S9 (Supporting Information) shows the rapid DOX loading property. The loading efficiency was 109% (w/w), which was larger than that of previous drug delivery systems (Table S2, Supporting Information). The combined efficiency of electrostatic and noncovalent interactions achieved a high DOX-loading capacity, including π–π stacking effects and hydrophobic interactions. In normal biological environments (pH 7.0), DOX in NPMOFs was released at a very slow rate (8% after 8 h and only 10.3% after 72 h), which ensured a high biocompatibility and low side effects to normal tissues. However, in cancerous conditions (pH 5.0, e.g., lysosome), a faster release was observed (34.3% after 8 h and 58.5% after 72 h) indicating a capacity for high therapeutic effects (Figure 2e). The pH-response release properties of DOX provided smart drug delivery for tumor-targeting treatment with our NPMOFs.
The cytotoxicity of NPMOFs was evaluated to validate their biocompatibility, PDT, and DOX-release efficiency using a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cell viability of HepG2 cells (a human liver cell line) was recorded after incubation with NPMOFs or DOX@NPMOFs for 8 h (Figure 2f). The PDT-related groups were irradiated with a 655 nm laser for 10 min (180 J cm\(^{-2}\)). The cell viability of the single-NPMOFs-treated group in all dosages was higher than 90% (Figure 2f black), indicating their high in vitro biocompatibility. The viability of PDT treated HepG2 cells was less than 60%, which confirmed the PDT efficiency of NPMOFs (Figure 2f red). The smart delivery of DOX@NPMOFs resulted in the chemotherapy group having a lower viability (22%) (Figure 2f blue). As expected, low IC\(_{50}\) (67.72 µg mL\(^{-1}\)) and high cell lethality (90%) were observed following cotherapy of DOX@NPMOFs under 655 nm laser irritation (Figure 2f purple).

Fluorescent imaging of NPMOFs to illustrate their potential as IGTSs was confirmed using HepG2 cells, zebrafish, and mice models. Cellular imaging was performed using a confocal laser scanning microscope (CLSM) on HepG2 cell lines after incubation with NPMOFs or DOX@NPMOFs for 8 h. The blue fluorescence in the cell nuclei was attributed to 4′,6-diamidino-2-phenylindole (DAPI), whereas the red emission was attributed to NPMOFs or DOX (Figure S10, Supporting Information). The merged images revealed that NPMOFs were only detectably present in the cytoplasm. However, the released DOX could penetrate the cell nucleus (Figure S11, Supporting Information). Therefore, the DOX-DNA complex prevented the proliferation of cancer cells.\(^{[21,46,47]}\) HepG2 cells incubated with NPMOFs were examined by CLSM imaging to validate the effect of incubation time and NPMOF concentration (Figures S11–S13, Supporting Information). The results validated that the size, morphology, and near-neutral zeta potential of NPMOFs achieved fast cell uptake for high cellular imaging efficiency.\(^{[34]}\)

Zebrafish is an important model organism for studying biological toxicity, in vivo system damage, and biocompatibility of nanoparticles and drugs.\(^{[48]}\) Bright field and multicolor fluorescence images of zebrafish after being soaked...
with NPMOFs were recorded at different concentrations (0–250 µg mL⁻¹ in Figures S14–S17, Supporting Information). Green fluorescence was observed as the autofluorescence of the zebrafish, while red fluorescence from NPMOFs showed a higher signal in the intestine (blue arrow in Figure S17, Supporting Information) and yolk sac (yellow arrow in Figure S17, Supporting Information). The differences in brightness between the yolk and other zebrafish tissues confirmed the different affinity of NPMOFs to the tissues. The distribution of NPMOFs was, therefore, clearly illustrated in zebrafish and validated the practicality of NPMOFs as imaging probes.

No malformation or lesion was detected throughout the whole growth period to confirm the low toxicity to zebrafish.

The distribution of NPMOFs was investigated by tracking fluorescent trajectory in a mouse model to prove the biocompatibility of NPMOFs-based IGTS in mammals. Figure 3 shows the absorption, distribution, metabolism, and excretion (ADME) process of NPMOFs. NPMOFs first accumulated in the liver and then entered the intestine to be excreted through dejecta. The ADME process of NPMOFs was similar to coproporphyrin but with a prolonged circulation time. The efficient circulation and clearance of NPMOFs validated their safety as an IGTS platform. Some NPMOFs spread to the lymphatic system from blood circulation, which further enhanced the retention time of NPMOFs. The lymph accumulation phenomenon was derived from the porphyrin and can be observed even in tumor-bearing mice (Figure 4 and Figure S18, Supporting Information). Cancer metastasizes mainly through the circulatory and lymphatic systems; thus, the ADME of our NPMOFs may provide a chance to block the metastasis of cancer through their chemotherapy and PDT capacities.

The objective of fluorescent imaging is to probe and monitor the tumor for imaging-guided therapy. In vivo fluorescence of NPMOFs was recorded in HepG2 tumor-bearing mice. Tumors were clearly labeled with high resolution and signal-to-noise ratio (Figure 4a and Figure S18, Supporting Information). The bright spot on the leg after 5 min injection was attributed to fast accumulation in a small lymph node (yellow arrow) at 5 min and in subcutaneous transplantable tumor (blue arrow) after 7 h through EPR effect. B) Fluorescent imaging of dissected organs of cancer-bearing mouse. C) Biodistribution of NPMOFs in main organs of mice (tumor, intestine, liver, stomach, heart, spleen, blood, and kidney).
postinjection (Figure 4b). That the highest fluorescent intensity was in cancer tissue indicated tumor-targeted delivery for imaging, PDT, and drug release. The fluorescence in the stomach and the intestine showed that NPMOFs were metabolized through the digestive tract. The biodistribution of NPMOFs was evaluated by ICP-MS after dosing for 7, 20, and 36 h (Figure 4c). The distribution of NPMOFs was in good agreement with the fluorescence imaging results. The content of Zr increased in the liver, spleen, kidney, and intestine but reduced in cancer and other tissues at 20 h, indicating NPMOFs were metabolized by the liver and kidney. The Zr content recovered to normal levels at 36 h, indicating that NPMOFs were cleaned rapidly. The cytotoxicity to organs was tested with histological analysis. No significant inflammatory reaction or histopathological changes were observed (Figure S19, Supporting Information). The pharmacokinetics of DOX was also tested. Most DOX accumulated in the tumor and maintained high concentrations, indicating the possibility of positive therapeutic effects, while the lower levels of DOX in the blood and heart ensured low cytotoxicity to normal tissues. All the results confirmed that NPMOFs did not cause in vivo damage to normal tissues and organs due to the high biocompatibility.

The visualization of drug delivery indicates that NPMOFs have advantages for cancer diagnosis and drug monitoring. Motivated by the excellent in vitro PDT and chemotherapy efficiency and high tumor accumulation of DOX@NPMOFs, in vivo cotherapy efficiency of DOX@NPMOFs was evaluated on HepG2 tumor-bearing mice. The efficiency of chemotherapy-and-PDT cotherapy of IGTS was tested with two single therapies and saline for comparison. The fluorescence intensity at the cancer site was first recorded by imaging. After the accumulation of NPMOFs reached a maximum, the mice of PDT-treated groups were irradiated at the tumor site with a laser at 180 J cm\(^{-2}\) for 15 min. The tumor sizes in the mice from different groups were subsequently monitored. The tumors of the PDT group and the chemotherapy group exhibited slower growth than that of the control (Figure 5a). However, the tumors of mice treated with DOX@NPMOFs began to shrink after 2 d of chemotherapy-and-PDT cotherapy. Most importantly, two tumors were completely eradicated, and another two tumors decreased from 62.5 to 2 mm\(^3\) among the four tumors in the DOX@NPMOFs cotherapy group after 10 d (Figure 5b-d). Therefore, DOX@NPMOFs were efficiently internalized by the tumor cells and induced cytotoxicity upon chemotherapy and PDT. No skin/tissue damage was observed after PDT treatment in any of the mice due to the long wavelength and low energy irradiation (Figure 5c).

We also found that the mice behaved normally and had no noticeable reduction in body weight after chemotherapy-and-PDT cotherapy with DOX@NPMOFs (Figure S21, Supporting Information). No appreciable organ damage or inflammatory lesions were seen in mice 10 d after DOX@NPMOFs-based cotherapy, as revealed by hematoxylin and eosin (H&E)-stained major organ slices of the mice (Figure 6a). Tumors of the mice in different groups were analyzed to illustrate therapy efficiency (Figure 6b). Saline-treated tumors (control group) showed explosive growth without any damage, while the PDT group had necrosis inside the tumor after being subjected to laser irradiation. The DOX@NPMOFs group had necrosis on the periphery of the tumor because of the antidrug effect of tumor cells. The chemotherapy-and-PDT cotherapy group showed the highest therapeutic efficiency, and necrosis was observed throughout the tumor. \(\text{H}_2\text{O}_2\) produced from PDT and DOX have a synergistic effect in the tumor tissue; thus, a high cotherapy efficiency was observed with the necrosis from the inside to the

Figure 5. a) Tumor growth inhibition curve after PDT (P), chemotherapy (C), and chemotherapy-and-PDT dual therapy (C+P) treatment. \(V_0\) and \(V\) refer to tumor volumes before and after treatment with the injection of NPMOFs. All of the mice were subjected with 10 mg kg\(^{-1}\) NPMOFs. b) Tumor weight after NPMOFs treatment for 10 d. c) Photos of the mice after treatment for 10 d. d) Photos of tumor in each group after treatment. Two tumors in DOX@NPMOFs for PDT and chemotherapy were completely eradicated after 10 d.

Figure 6. a) Histological analysis of main organs. b) Histological analysis of tumor tissue. The black arrow points to the necrosis part of tumor. Saline-injection (Control) group has no damage in tumor; NPMOFs for PDT (P) group caused necrosis inside the tumor; DOX@NPMOFs for chemotherapy (C) group caused necrosis on the periphery of tumor; and DOX@NPMOFs for chemotherapy-and-PDT (C+P) group showed the highest therapy efficiency that necrosis was observed all over the tumor.
periphery of the tumor with DOX@NPMOFs. Thus, DOX@NPMOFs showed high biocompatibility to the main organs and high therapy efficiency to the tumor tissue as a smart IGTS (Figure 6).

3. Conclusions

In summary, we succeeded in building an NPMOF-based IGTS to label and treat cancer simultaneously with chemotherapy-and-PDT cotherapy afterward. The ADME process of NPMOFs in mice revealed their selective accumulation at the cancer site. In combination with selective laser irradiation and smart DOX release, the tumor tissue was efficiently treated, while fewer side effects were observed in normal tissues. Therefore, NPMOFs showed a high biocompatibility for imaging-guided chemotherapy and PDT cotherapy with a high safety and treatment efficiency. The biocompatibility with normal tissues and cytotoxicity to tumor tissue indicate that NPMOFs represent an effective probing and therapy system for potential translation to clinical applications. Our work validated that MOFs are an ideal IGTS material because of their flexible design, easy-to-integrate functional building blocks, and porous structure.

Supporting Information

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

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