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Nanoscale metal–organic frameworks for combined photodynamic & radiation therapy in cancer treatment

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ABSTRACT

Nanoscale metal organic frameworks (NMOFs) have shown great potential in biomedicine owing to their structural/chemical diversities, high molecular loading capacities, and intrinsic biodegradability. Herein, we report the rational design of a NMOF composed by hafnium (Hf^{4+}) and tetrakis (4-carboxyphenyl) porphyrin (TCPP). In such Hf-TCPP NMOFs, while TCPP is a photosensitizer to allow photodynamic therapy (PDT), Hf^{4+} with strong X-ray attenuation ability could serve as a radio-sensitizer to enhance radiotherapy (RT). Those NMOFs with polyethylene glycol (PEG) coating show efficient tumor homing upon intravenous injection, and thus could be used for in vivo combined RT & PDT, achieving a remarkable anti-tumor effect. Importantly, Hf-TCPP NMOFs show efficient clearance from the mouse body, minimizing concerns regarding their possible long-term toxicity. Our work thus presents a new concept of developing multifunctional NMOFs as a biodegradable carrier-free system, in which both metal ions and organic ligands are fully utilized to exert their therapeutic functions.

1. Introduction

Radiation therapy (or radiotherapy, RT) that employs ionizing irradiation such as X-ray to destruct cancer cells is an extensively used cancer treatment method in the clinic. RT can be used for primary therapy of localized solid tumors, or as adjuvant and palliative therapies to relieve symptoms from later stage or metastatic cancers [1–3]. Therefore, it would be clinically meaningful to explore effective approaches to enhance the efficacy of RT and reduce its side effect to normal organs. For this purpose, nanoparticles containing high-Z elements (e.g. Au [4], Hf [5], Bi [6] and rare earth elements [7–10]), which are able to interact with ionizing radiation to produce photo/auger electrons and thereby generate reactive free radicals to destruct cancer cells [10,11], have shown promises to sensitize RT-induced tumor killing. However, an important disadvantage of those inorganic nanoparticles as radiosensitizers is that usually they are not biodegradable and would exhibit long-term body retention, which may raise long-term toxicity concerns and greatly limit their potential in clinical translation. Meanwhile, how to combine RT with other commonly used therapeutic approaches utilizing multifunctional RT-sensitizing nanoparticles would be of great interests in the future development of new RT-based cancer treatment strategies [7,8,11–13].

Metal organic frameworks (MOFs) are a class of hybrid materials formed by the self-assembly of metal ions or clusters and organic polydentate bridging ligands [14,15]. Due to virtually limitless combinations of metals and ligands, the physicochemical properties of MOFs can be judiciously tuned for a large variety of promising applications [16–19]. Recently, MOFs have been scaled down to the nanoscale, forming nanoscale MOFs (NMOFs) which not only maintain the structural diversity and physicochemical properties like bulk MOFs, but also exhibit potential applications in biomedicine such as nanocarriers for loading of imaging agents and drug

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molecules [20–24]. Nanoscale dimensions of NMOFs allow them to take advantage of the enhanced permeability and retention (EPR) effect of cancerous tumors to achieve enhanced tumor accumulation, useful for cancer-targeted imaging or treatment [14,25,26]. To date, many anti-cancer drugs such as cisplatin [25,27,28], doxorubicin [21], methotrexate [29], 5-fluorouracilor [30–32] and nucleic acids [33,34] have successfully been incorporated into NMOFs to realize chemotherapy and gene therapy. Furthermore, photosensitizers integrated NMOFs have also been developed for photodynamic therapy (PDT) by generating singlet oxygen ($^1O_2$) under light exposure [35,36]. However, while the therapeutic functions of NMOFs are realized mostly through the incorporated organic ligands or loaded drug molecules [37], little attention has been paid to the utilization of metal ions in the NMOF structure to achieve or enhance therapeutic performances. In particularly, the application of NMOFs, many of which contain high-Z metal ions with strong X-ray attenuation capabilities, to enhance RT cancer treatment, has not yet been reported to our best knowledge.

Herein, NMOF nanoparticles are synthesized based on hafnium ions (Hf^{4+}) and tetrakis (4-carboxyphenyl) porphyrin (TCPP) [38], the latter of which is a commercially available photosensitizer used in PDT. We choose Hf ions as the radiosensitizer to contrast NMOF for the following reasons: (1) Hf as a high-Z element is able to interact with ionizing radiation to produce photo/auger electrons and thereby generate reactive free radicals to destruct cancer cells [5]. (2) Hf shows strong binding ability to coordinate with many organic ligands to form nanoscale MOFs [35,36]. (3) Hf has been reported to be a relatively safe agent without significant biotoxicity [39]. In the meanwhile, the symmetrical molecular structure of TCPP and the strong coordination ability of its four carboxyl groups to metal ions could facilitate the formation of MOF structure. After polyethylene glycol (PEG) modification, the obtained PEGylated NMOF (NMOF-PEG) nanoparticles show excellent stability in physiological solutions. It is found that such NMOF-PEG nanoparticles with an unprecedentedly high photosensitizer (TCPP) loading appear to be extremely effective in producing singlet oxygen under light exposure for PDT. Meanwhile, the high-Z element Hf^{4+}could act as a radiosensitizer by absorbing the ionizing radiation to enhance RT efficacy. Upon intravenous injection, those NMOF-PEG nanoparticles show efficient tumor retention at the result of EPR effect. The in vivo combination therapy with NMOF-PEG injected tumor-bearing mice is then designed by applying PDT 8 h post RT, so that the tumor oxygenation would be largely recovered, achieving a great therapeutic effect in preventing tumor growth. Importantly, it is further uncovered that such NMOF-PEG nanoparticles, without showing obvious toxicity to the treated animals, would be degraded and then excreted in a relatively rapid manner, with the majority of Hf^{4+} cleared out in 7 days. Our work presents the great promises of NMOFs as a biodegradable multifunctional tumor-homing therapeutic platform for cancer treatment.

2. Experimental section

2.1. Materials

All chemicals, unless specified otherwise, were purchased from Sigma-Aldrich and used as received. Tetrakis (4-carboxyphenyl) porphyrin (TCPP) was purchased from TCI, Shanghai, China. Hafnium (IV) tetrachloride ($HfCl_4$) was purchased from Alfa Aesar. PEG polymers were purchased from PegBio, Suzhou, China. All cell-culture related reagents were purchased from Hyclone.

2.2. Synthesis of Hf-TCPP NMOF nanoparticles

Firstly, 2 ml of $HfCl_4$ solution [2 mg/ml in *N,N*-dimethylformamide (DMF)], 2 ml of TCPP solution (5 mg/ml in DMF), and 0.4 ml of acetic acid were added to a 20 ml glass vial. Then the reaction mixture was kept in an 80 °C oven. After 2 h, 6 ml DMF was added into the reaction system for another 24 h. The dark purple solid product was collected by centrifugation and washed with DMF, triethylamine/ethanol (v/v = 1:20) and ethanol, subsequently. Finally, the purified NMOF nanoparticles were re-dispersed in chloroform for further use.

2.3. Surface modification of Hf-TCPP NMOF nanoparticles

C18PMH-PEG polymer was synthesized according to a previous report [40]. 5 ml of NMOF (Hf-TCPP) chloroform solution was mixed with 200 μl chloroform containing 5 mg C18PMH-PEG and stirred overnight at room temperature. After slow evaporation of solvent under low-pressure, a dry film of nanoparticles was obtained and then dispersed into distilled (DI) water. To remove the excess polymer, the solution was washed with DI water repeatedly, obtaining purified NMOF-PEG sample which was re-dispersed in DI water.

2.4. Characterization

Transmission electron microscopy (TEM) and high-resolution TEM (HR-TEM) images were obtained using a Philips CM300 transmission electron microscope operating at an acceleration voltage of 200 kV. UV–vis–NIR absorption spectra were recorded with a GENESYS 10S UV–Vis spectrophotometer. The hydrodynamic diameters of NMOF and NMOF-PEG nanoparticles were determined by a Zetasizer Nano-ZS (Malvern Instruments, UK). The Brunauer-Emmett-Teller (BET) surface area and pore size were measured using ASAP2050 system. Thermo-gravimetric-differential thermal analysis (TG-DTA) measurements of the products was performed using a Setaram TGA 92 instrument in the temperature range from room temperature to 700 °C at a heating rate of 3 °C/min. Concentrations of Hf^{4+} were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

2.5. Cellular experiment

4T1, HeLa, and NIH3T3 cells originally obtained from American Type Culture Collection (ATCC) were cultured at 37 °C under 5% CO_2. All cell culture related reagents were purchased from Invitrogen. Confocal fluorescence imaging of cells was performed using a Leica SP5 laser scanning confocal microscope.

2.6. In vitro radiation therapy

For clonogenic assay, 4T1 cells were cultured in 6-well plates and incubated at 37 °C for 24 h. After 6 h of incubation without or with Hf-TCPP NMOF-PEG (80 mg/L), the cells were irradiated by X-ray with different radiation doses (0, 2, 4, 6 and 8 Gy). Afterwards those cells were washed with PBS and further incubated in fresh cell culture medium at 37 °C for 7 days, before they were fixed with anhydrous methanol and stained with Crystal violet (CV, Sigma-Aldrich). The resulting colonies were counted only if they contained more than 50 cells. The surviving fraction = (surviving colonies) / (cells seeded × plating efficiency). The mean surviving fraction was obtained from three parallel tests.