Functionalized zeolitic imidazolate framework-8 as a drug carrier for bioimaging

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Abstract. There has been a surging interest in the synthesis of multifunctional nano platform for drug delivery and bioimaging. ZIF-8 of the zeolitic imidazolate series is widely used in the biological field as the leader of metal-organic frameworks (MOFs). Herein, we processed ZIF-8 nanoparticles (ZIF-8 NPs) by pyrolysis with high temperature, while ensuring that it does not destroy itself. The strategy of calcination ZIF-8 NPs at 600℃ for 5 min has a higher loading rate for doxorubicin hydrochloride (DOX). Moreover, through bioimaging of ZIF-8@C in A549 lung cancer cell, the red fluorescence was produced, indicating that ZC-6005@DOX successfully entered the cancer cell.

1. Introduction
At present, an army of anticancer drugs enjoy achieved better results in clinical application, but mainly focus their adverse bio-distribution in the cell, damage to normal tissue, affecting its application and efficacy[1]. High doses of drugs are often needed to compensate for adverse biological distributions for therapeutic purposes, and dose-related side effects are often present in chemotherapy[2]. In the process of looking for the best technique, nanomaterials can effectively control normal tissue damage and provide a rosy opportunity for biomedical.

Metal-organic frameworks (MOFs) have been demonstrated as a novel class of multifunctional nanomaterials or precursors for applications in gas storage and separation[3], catalysis[4], and chemical sensing[5] because of the unique properties of MOFs such as their ultra-high porosity and tunable functionality, [6-9] At present, the biomedical application of traditional MOFs has also attracted much attention due to the high surface area and excellent biocompatibility[10]. yet the physical environment is complex in the human body, which is far from enough to achieve considerable drug delivery in such complicated environment only by using traditional MOF materials. Post-manufacture modification can serve as a perfect option to achieve additional functionalities for MOFs, especially for nanocarbons derived from MOFs.

To achieve high-performance loading capability of DOX, herein, we put forward a strategy to prepare emerging MOF@C composite by short-term and high-temperature calcination of ZIF-8 NPs. The composite material can be used in bioimaging and anticancer therapy.

2. Experimental section

2.1 Materials and measurements
All solvents and reagents for the synthesis were purchased from commercial sources and used as received unless otherwise indicated. Aqueous solutions were prepared with deionized water (18.2 MΩ cm) produced from a Milli-Q water purification system. Transmission electron microscopy (TEM) images were collected on a JEM 2100F instrument at an accelerating voltage of 200 kV. Ultraviolet-visible (UV-Vis) spectra were recorded on Cary 50 instrument from American Varian Company. PXRD measurements were recorded ranging from 5° to 40° at room temperature on a Siemens D5005 diffractometer with Cu Kα (λ = 1.5418 Å). Fluorescence microscopy images were observed on a EVOS XL Core (Thermo). A549 cells (human non-small cell lung cancer cells) were provided by the American Type Culture Collection and were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% FBS.

2.2 Synthesis of ZIF-8, ZIF-8@C and ZC@DOX

2.2.1 Synthesis of ZIF-8 NPs

ZIF-8 nanoparticles (100 nm) were fabricated according to the devised a protocol with slight modification[11]. Briefly, ZIF-8 NPs were produced by the addition of methanol solution (16 mL) of Zn(NO$_3$)$_2$·6H$_2$O (0.5 mmol) and 2-methylimidazole (2 mmol) under stirring for 2 h at 40°C. The white solids were collected by centrifugation and washed several times with methanol.

2.2.2 Synthesis of ZIF-8 derived carbon nanoparticles

ZIF-8@C nanoparticles were constructed by direct pyrolysis of the ZIF-8 NPs under a flow of nitrogen gas. Briefly, the ZIF-8 NPs sample was dissolved in methanol in a ceramic boat and transferred into a tube furnace. Then, the sample was heated to 600°C and maintained for 1 min, 3 min, 5 min, 30 min, 60 min under flowing N$_2$. Upon cooling to room temperature, the ZIF-8@C NPs were fabricated.

2.2.3 Dox Loading

To incorporate DOX into the pores of ZIF-8@C nanoparticles, the nanoprecipitation method with a little modification was adopted. 10 mg DOX was dissolved in 5 mL of distilled water (DI water), and then 10 mg of freshly dried ZIF-8@C NPs was dispersed in DOX solution. After stirring in the dark for 48 h, the nanoparticles were recovered by centrifugation with 10000 rpm for 15 min.

2.2.4 Cell culture and fluorescence imaging

A549 cells (human non-small cell lung cancer cells) were provided by the American Type Culture Collection. Cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) and 10% fetal bovine serum (FBS) with 5% CO$_2$ at 37°C. For imaging studies, A549 cells (1 × 10³ cells/well) were passed onto the culture dishes and incubated for 12 h, then the ZC-DOX was added and incubated with A549 cells. After 8 h and 20 h, washing the culture dishes three times with PBS, fluorescence imaging experiments were carried out on a EVOS XL Core fluorescence microscope (Thermo Fisher).

3. Results and discussion

The synthesis procedure of the ZC-DOX NPs is shown in Scheme 1. Since a significant weight loss of ZIF-8 NPs at 600°C as evidenced by TGA curves[12]. We thus select 600°C for subsequent treatment.
Scheme 1. Schematic illustration of the combinational drug delivery platform based on ZIF-8 derived carbon nanoparticles (ZC@DOX).

The ZIF-8@C nanoparticles exhibit are fabricated by pyrolysis with high temperature of the ZIF-8 NPs under a flow of nitrogen gas. The ZIF-8 nanoparticles and ZIF-8 derived carbon nanoparticles exhibit a typical sodalite morphology in the transmission electron microscopy (TEM) are shown in Fig. 1(a-d) with a diameter of approximately 100 nm. Compared with smooth surfaces of ZIF-8 NPs, there are several small tunnels on the surfaces of ZC-6005 NPs (Fig. 1d), indicating that ZC-6005 NPs are composed of two parts: ZIF-8 NPs and embedded carbon nanoparticles.

Fig. 1. (a) TEM images of ZIF-8 NPs, (b) calcination ZIF-8 NPs at 600°C for 1 min (ZC-6001 NPs), (C) 3 min (ZC-6003 NPs), (D) 5 min (ZC-6005 NPs), (E) and (F) 30 min (ZC-6030 NPs).

The ZC-6030 NPs exhibit subsidence damage compared with the sodalite structure formed of ZIF-8 nanoparticles are shown in Fig. 1(e,f).
Fig. 2. PXRD patterns of ZIF-8 NPs with simulated (black line), as-synthesized ZC-6001 (red line), as-synthesized ZC-6003 (yellow line), as-synthesized ZC-6005 (blue line) and as-synthesized ZC-6030 (green line).

The PXRD pattern of ZIF-8 NPs with pyrolysis 600°C for 5 min is illustrated in Fig. 2. It is evident that the diffraction peaks of ZC-6005 NPs specimen are in outstanding agreement with the simulation of ZIF-8 NPs, revealing the typical sodalite structure of ZIF-8 NPs without any impurities and residues. Furthermore, the well-defined sharp peaks indicate the excellent crystallinity of the ZIF-8 NPs.

Fig. 3. UV-Vis absorption spectra of DOX. Stock solution (red line); the supernatant after interacting with ZC-6005 NPs (black line) (DOX concentration = 2 mg mL⁻¹).

The entrapment of drugs into the ZC-6005 NPs is confirmed by absorption measurements (485 nm, a characteristic absorption of DOX)[13]. As shown in Fig. 3, after adding activated ZIF-8 NPs material to the aqueous solution of DOX (2 mg mL⁻¹), the intensity of the absorption peak of DOX is significantly reduced. This phenomenon shows that the content of DOX in the solution has been reduced, indicating that ZIF-8 NPs has a storage capacity for DOX.

The DOX uptake is directly observed by EVOS XL imaging system. ZC-6005 NPs as nanoscale material is infiltrated by the A549 cells via an endocytosis process. As illustrated in Fig. 4, the accumulation amounts of DOX (red fluorescence) are located in the cytoplasm of A549 cells after incubating A549 cells with ZC-6005 NPs for 20 h.

Afterward, the cytotoxicity of ZC-6005 NPs was evaluated is shown in Fig. 5. The cell viabilities of the A549 cells were all over 80% at the tested concentrations from 5 to 50 μg mL⁻¹ after incubation.
with ZC-6005 NPs for 24 h indicating good biocompatibility and negligible cytotoxicity of ZC-6005 NPs.

Fig. 4. Fluorescence images of A549 cells incubated with ZC-6005@DOX. Images with bright field and incubated with ZC-6005@DOX for 8 h (a), 20 h (b) (DOX concentration = 5 μg mL⁻¹). Scale bar: 200 μm.

4. Conclusions
To sum up, we investigate a novel kind of pH-responsive coordination nanocarbons (ZC-6005 NPs) with superior stability for drug delivery and imaging by a simple coordination method. Meanwhile, in vitro experiments confirm the ZC-6005 NPs possess negligible cytotoxicity. Thus, the novel ZIF-8 derived carbon nanoparticles are expected to possess widely used in the drug delivery of cancer, and imaging capability can be exceedingly useful in designing and developing of novel materials with higher performances for biomedical applications.

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