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Preparation of spherical metal–organic frameworks encapsulating ag nanoparticles and study on its antibacterial activity

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ABSTRACT

A metal–organic frameworks (CuTCPP MOFs) were synthesized with $Cu(NO_3)_2 \cdot 3H_2O$ and 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin (TCPP) by the solvothermal method. The structure and morphology of the CuTCPP MOFs were characterized by UV–vis absorption spectra, X-ray diffraction (PXRD), energy dispersive spectra, scanning electron microscopy (EDS-SEM) and transmission electron microscopy (TEM). The structure of the as-synthesized MOF includes copper ions and copper metalloporphyrin (Cu-TCPP) by UV–vis absorption spectra and PXRD. The SEM and TEM images of the as-synthesized MOF showed the morphology of the CuTCPP MOFs were spherical. The as-synthesized spherical MOFs as the carriers were used to encapsulate the Ag nanoparticles and prepared Ag-CuTCPP MOFs. The Ag-CuTCPP MOFs was also characterized by UV–vis, PXRD, SEM and TEM. The Ag nanoparticles were completely encapsulated into the CuTCPP MOFs and no surface absorption, which have been confirmed by comparing TEM and SEM-EDS of Ag-CuTCPP MOFs before crushing with that of Ag-CuTCPP MOFs after crushing. In addition, the release of Ag ions from Ag-CuTCPP MOFs was also investigated by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Furthermore, the antimicrobial activities and cytotoxicity of Ag-CuTCPP MOFs were performed by *in vitro* and *in vivo* experiment. *In vitro*, the antibacterial effect of Ag-CuTCPP MOFs was even better than that of the penicillin as the positive control and the cytotoxicity of Ag-CuTCPP MOFs was significantly lower than that of naked Ag nanoparticles and Ag ions; *in vivo*, Ag-CuTCPP MOFs not only exhibited the excellently antibacterial effect and extremely low cytotoxicity but also effectively promoted the wound healing.

1. Introduction

The bacteria have dominated on our planet for more than two billion years and they have successfully outlasted or evolved from many obstacles which they have encountered. The development trend of the bacteria had been effectively controlled until the discovery of antibacterial drugs in the 20th century [1,2]. However, most of the antibacterial drugs have the specific intracellular targets, which have lead that the bacteria could progressively develop compensatory mechanisms to resist the antibacterial drugs and formed multi-drug resistant bacteria. The multi-drug resistant bacteria caused the infectious diseases which have fiercely threatened to human health [1–5]. Most seriously, the evolution of multi-drug resistant bacteria has outpaced the development of antibacterial drugs in the past few decades [1–3]. In order to effectively resolve the multi-drug resistant bacteria, many researchers have committed to finding some new antibacterial agents which not only effectively control multi-drug resistant bacteria but also stand the test of time. Among these new antibacterial agents, the nanomaterials have demonstrated excellent antibacterial ability because they have the unique chemical and physical properties, nanometer effects and the high surface area to volume ratio. Ag nanoparticles as the ideal antibacterial nanomaterials substituted for the traditional antibiotics, which have attracted many researchers' interest due to its broad-spectrum of antimicrobial activities which suppressed both Gram-positive and Gram-negative bacteria, eukaryotic microorganisms and viruses [6–12]. Generally, the antibacterial activity of Ag nanoparticles has been ascribed to the release of Ag ions and the direct contact with bacterial cells. Furthermore, the bacteria could hardly produce the resistance to Ag nanomaterials due to the antibacterial mechanisms of Ag nanomaterials. However, the excessive release of Ag ions would be harmful to the normal tissues and Ag itself could cause agyrosis and argyria because of the long-term direct contact between Ag and the normal tissues [13–15]. An outstanding way to resolve this problem is to choose an effective carrier which not only maintain the antibacterial activity of the Ag nanoparticles but also prevent the excessive release of Ag ions and avoid the long-term direct contact between Ag and the normal tissues [16–19].

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The metal-organic frameworks (MOFs) could act as the carrier of Ag nanoparticles because MOFs were a class of hybrid materials which possess the large surface area, highly regularized pores and the ordered crystalline structure [20,21]. MOFs also exhibit some desired characteristics as ideal potential drug carriers including the connatural biological degradation ability and drug loading. Furthermore, the structural, morphology and physicochemical properties of MOFs could be tuned by refining the organic linkers, metal connecting points and reaction conditions, which provide an enormous flexibility for the application of MOFs in drug delivery [22–29].

Porphyrin and metalloporphyrin as the bioactive ingredient have been used to construct a variety of biomaterials because of their unique chemical, physical and biological functionalities. The variety of functional group substituents in the periphery of the porphyrin molecule and the various derivatives combining different center-coordinated metals allowed the fine control of connection motif, porphyrin and metalloporphyrin molecules as outstanding functional ligands have been used to construct novel MOFs materials [30–34]. The novelty and functional diversification of MOFs thin films have been synthesized based on Cu-TCPP and copper ions [20,26]. The MOFs based on Cu-TCPP and copper ions have attracted considerable attention for their application in photocatalysis, sensing, photocurrent generation and conduction [30–34]. In addition, the MOFs based on porphyrin derivatives could have the excellent biocompatibility by selecting the ideal metals ions with good biocompatibility as the metal nodes of the MOFs. The MOFs based on the Cu-TCPP and copper ions have the possibility of good biocompatibility due to the copper as an important inherent trace element in the body. However, up to date, the application of CuTCPP MOFs as drug delivery materials has not still been reported, which could be attributed to the followed reasons: The ideal drug-carrier materials must hold the regular morphology, high drug loading content, relatively easy-to-fabricate and appropriate diameter-distribution aside from the single component and the clear structure. To our knowledge, the morphology of the present CuTCPP MOFs reported was only nanofilms and nanosheets, which was difficultly used as the ideal drug-carrier materials to encapsulate drugs [35–38].

In this paper, A CuTCPP MOFs materials were successfully synthesized with $Cu(NO_3)_2 \cdot 3H_2O$ and TCPP by using the solvothermal method. The morphology and size-distribution of the CuTCPP MOFs was well controlled. Furthermore, the CuTCPP MOFs was successfully used to encapsulate the Ag nanoparticles and prepared the new composite materials Ag-CuTCPP MOFs. The Ag nanoparticles were completely encapsulated into the CuTCPP MOFs and no surface absorptions by comparing TEM and EDS of Ag-CuTCPP MOFs before crushing with that of Ag-CuTCPP MOFs after crushing. In addition, the release of Ag ions from Ag-CuTCPP MOFs was also investigated by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Furthermore, the antimicrobial activities and cytotoxicity of Ag-CuTCPP MOFs were performed by *in vitro* and *In vivo* experiment. *In vitro*, the antibacterial effect of Ag-CuTCPP MOFs was even better than that of the penicillin as the positive control and the cytotoxicity of Ag-CuTCPP MOFs was significantly lower than that of naked Ag nanoparticles and Ag ions; *in vivo*, Ag-CuTCPP MOFs not only exhibited the excellently antibacterial effect and extremely low cytotoxicity but also effectively promoted the wound healing.

2. Experimental

2.1. Materials

TCPP were purchased from Klamar (97%, Shanghai Co., Ltd.). Polyethylenimine (PEI, low mol. wt., 50 wt% solution in water) and silver nitrate($AgNO_3$) were from Sigma Trading Co., Ltd. (Shanghai), $Cu(NO_3)_2 \cdot 3H_2O$, ethanol, DMF, nitrate acid and hydrofluoric acid (HF, 40%) were purchased from Fuyu Fine Chemical Co., Ltd. All reagents are of analytical purity. The ultra-pure water (>18 MΩ * cm) was used in this study. The *Escherichia coli (ATCC8739)*, *Staphylococcus aureus (ATCC 6538)* and *Bacillus subtilis (ACCC 11060)*, were from Heilongjiang Provincial Academy of Sciences Institute of Applied Microbiology (Harbin, China). They were refreshed in the LB nutrient broth at 37 °C, and cultivated to mid-log phase prior to the *in vitro* antimicrobial experiments. Female Kunming mice (KM) were purchased from the laboratories of The 2nd Affiliated Hospital of Harbin Medical University and studied the age between 4 and 5wk of age. All procedures were approved by the University Ethics Committee of the Harbin Institute of Technology.

2.1.1. Synthesis of CuTCPP MOFs [20,30,32]

The CuTCPP MOFs were synthesized by using a mixture of TCPP (20 mg, 0.025 mmol) and $Cu(NO_3)_2 \cdot 3H_2O$(100 mg, 0.139 mmol) in 9 mL of mixed solvent (DMF:ethanol: pure water = 1:1:1), followed by the addition of 9 mL nitric acid (1 mol/L) into reaction system. The solution was stirred for at least 5 min at room temperature, and then the solution was transferred into a 20 mL Teflon-lined autoclave, sealed and maintained at 85 °C for 48 h. The synthesized powder was collected by centrifuging and washed with DMF, H_2O and ethanol, respectively. After dried at room temperature, a deep red powder was obtained. Elem. Anal. Cal (%) for the product: C, 58.04; H, 2.64; N, 5.64. Found: C, 58.64; Cu, 19.72; N, 5.81.

2.1.2. Synthesis of Ag-CuTCPP MOFs

2.1.2.1. Synthesis of Ag nanoparticles. The Ag nanoparticles were synthesized using a solvothermal reaction as the method in our early report, the synthetic procedure was as followed: 5% of PEI water dilute solution (1 mL) and 1% $AgNO_3$ solution (0.5 mL) were added in a beaker, added into 15 mL of pure water, stirred for 30 min at the room temperature, added hydrofluoric acid to adjust the solution pH to 4.0, adjusted to obtain a 20 mL solution with pure water, finally the mixed solution was heated to 140 °C for 4 h. The prepared Ag colloid solution was centrifuged at 3000 g for 10 min and washed twice with the pure water, then removing the supernatants and the nanoparticles were diluted by 6 mL pure water (approximately equal to 0.628 mg/mL).

2.1.2.2. Synthesis of Ag-CuTCPP MOFs. The Ag-CuTCPP MOFs was synthesized by repeating the above experimental procedure of CuTCPP MOFs. Only 3 mL Ag nanoparticles suspensions (0.628 mg/mL) replace the pure water in the above experimental procedure. The deep red powder Ag-CuTCPP MOFs were obtained.

2.2. Methods

UV–vis absorption spectra were measured by using UV-4000 spectrophotometer (VARIAN). The morphology of the CuTCPP MOFs and Ag-CuTCPP MOFs were observed by TECNAI G2 TEM and QUANTA FEC 250 SEM. The composition analyses were carried out by using QUANTA FEC 250 EDS. Powder X-ray diffraction (PPXRD) data were recorded on a PANalytical Empyrean using Cu-Kα radiation (λ = 1.5406 Å). The tissues sliced of the traumatic skin were observed using LEICA DM 2500 microscope. Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Optima 8300) was used to detect the release of Ag ions from Ag-CuTCPP MOFs and the analytical line of Ag was 328.068 ng.

3. Results and discussion

3.1. UV–vis absorption spectra of the CuTCPP MOFs and Ag-CuTCPP MOFs

UV–vis absorption spectra of TCPP, Ag nanoparticles, the CuTCPP MOFs materials and Ag-CuTCPP MOFs were measured and the results were displayed in Fig. 1. The UV–vis absorption spectrum of TCPP displayed the typical free-base porphyrin absorption bands includes an intense near-UV Soret absorption band at 419 nm and four visible