DRUG DELIVERY
2021, VOL. 28, NO. 1, 372–379
https://doi.org/10.1080/10717544.2021.1879316

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Enrofloxacin/florfenicol loaded cyclodextrin metal-organic-framework for drug delivery and controlled release

Yucai Wei[a], Chaoxi Chen[a], Shuo Zhai[a], Min Tan[a], Juebo Zhao[a], Xiaowen Zhu[a], Lu Wang[a], Qun Liu[a] and Tao Dai[b]

[a]College of Animal Science and Veterinary Medicine, Southwest Minzu University, Chengdu, China; [b]College of Chemistry & Environment, Southwest Minzu University, Chengdu, China

ABSTRACT

We presented an antibiotic-loaded γ-cyclodextrin metal-organic framework that delivered antibiotics suitable for the treatment of bacterial infections. The γ-cyclodextrin metal-organic framework was developed using γ-cyclodextrin and potassium ion via the ultrasonic method. The antibiotic (florfenicol and enrofloxacin) was primarily encapsulated into the pore structures of γ-CD-MOF, which allowed the sustained release of antibiotics over an extended period of time *in vitro* and *in vivo*. Notably, antibiotics-loaded γ-CD-MOF showed much superior activity against bacteria than free antibiotics (lower MIC value) and displayed better long-lasting activity (longer antibacterial time). The antibiotics-loaded γ-CD-MOF showed nontoxic and perfect biocompatibility to mammalian cells and tissues both *in vitro* and *in vivo*. These materials thus represent a novel drug-delivery device suitable for antibiotic therapy. This research is of great significance for reducing the generation of bacterial resistance and providing new ideas for the application of γ-CD-MOF.

ARTICLE HISTORY
Received 18 December 2020
Revised 15 January 2021
Accepted 18 January 2021

KEYWORDS
γ-Cyclodextrin; metal-organic framework; antibacterial ability

1. Introduction

To increase animal production, kinds of antibiotics have been used as veterinary drugs for the treatment of bacterial infections. Nevertheless, the drug residue had a long half-life and was difficult to eliminate in animal tissue, which was harmful to human health. At the same time, many bacteria had drug-resistant and the most commonly used antibiotics lost the effectiveness. Be faced with antibiotics resistance, the treatment of bacterial infections with antibiotics was a challenging task. Therefore, one of the efficient ways was to search for new conventional antibiotics. The new conventional antibiotics could temporarily improve the drug-resistance. However, the development of new antibiotics could not fundamentally solve the problem and the bacteria would show new drug resistance after long-term use (Andersson & Hughes, 2011; Tian et al., 2015). Therefore, it is urgent to exploit the way that could mitigate drug resistance and improve the efficacy. The nanotechnology could improve the therapeutic index at infection sites, achieve the controlled release of antibiotics and consequently decrease the dosage of drug administration (Xiong et al., 2014).

Nanotechnology was already largely considered and explored in many fields, especially in drug delivery systems. More and more researchers focused on nano-drug delivery (Nathan et al., 2005; Radovic-Moreno et al., 2012; Brooks et al., 2013; Atkinson et al., 2016; Liu et al., 2018; Anand et al., 2020). Yang and his coworkers (Liu et al., 2018) reported supramolecular nanofibers to deliver the antibiotic piperacillin/tazobactam through ionic interaction. The nanofibers demonstrated high drug loading efficiency and the release of PT from the nanofibers was sustained over 32 h. The drug-loaded nanofibers had remained antimicrobial activity toward both Gram-positive and Gram-negative bacteria. Importantly, in *Pseudomonas aeruginosa*-infected mouse skin wound model, the treatment with the drug-loaded nanofibers was more effective than free piperacillin/tazobactam for wound healing. These nanofibers are used as nanocarriers to deliver anionic antibiotics for the treatment of infections with improved efficacy. Amanda E. Brooks' group (Brooks et al., 2013) reported that polycaprolactone (PCL) polymer/antibiotic were coated over allograft bone void filler and allograft bone can act like a local, controlled drug release matrix in bone sites. Bacterial killing activity *in vitro* was demonstrated efficacious out to a clinically relevant 8-week time point. This combination device provides osteoconductive potential in bone voids while mitigating the potential for opportunistic infectious complications. In the above literature, the nanocarriers were constructed by the polymers and administered by skin-delivery. Mesoporous materials could also be used as antibiotic delivery materials. Biswanath Kundu's group (Anand et al., 2020) constructed the

CONTACT Tao Dai tdaicat@163.com College of Chemistry & Environment, Southwest Minzu University, No. 16, South Section, 1st Ring Road, Chengdu, Sichuan 610041, China

Supplemental data for this article can be accessed here.

mesoporous bioactive glass nanopowders by a wet chemical process, which could be loaded with ceftriaxone and sulbactam sodium (CFS), and loading efficiency was found to be ~40.4%. The drug-loading mesoporous bioactive glass was significantly able to inhibit both pathogens (*Staphylococcus aureus* and *Escherichia coli*). M.Zaharescua (Atkinson et al., 2016) prepared a kind of mesoporous bioactive glasses in $70SiO_2$-(26-x) CaO-$4P_2O_5$-xZnO by sol-gel method. The mesoporous bioactive glasses with 5% ZnO indicated good antibacterial activity and the inhibition rates could reach 91.3% for *Bacillus subtilis* and respectively 89.4% for *P. aeruginosa* after 2 h of incubation. Furthermore, Mark R. Towler and his coworkers (Nathan et al., 2005) reported that the gallium ion (Ga^{3+})-doped mesoporous bioactive glasses could not only exhibit antibacterial properties but also accelerate the blood coagulation cascade. These studies demonstrated that these mesoporous materials can be a potent candidate for drug-delivery and wound infection.

The metal-organic framework (MOF) is a novel porous crystal material with a reticulated backbone structure through the combination of metal ions or clusters and organic ligands (Hou et al., 2020). As a new porous material, MOF has many advantages especially its ultra-high porosity and specific surface area (Doan et al., 2019; Kaur et al., 2019). Based on these characteristics, MOF could be widely used in photoelectrocatalysis, gas storage, and separation (Zhang et al., 2016; Hilal & Seda, 2018; Lin et al., 2020; Yang et al., 2020). At the same time, it attracted more and more research in the field of biology. Wang Faming et al. found that MOF can be used as a vaccine carrier and cause the body to produce strong cellular and humoral immunity (Liu et al., 2020). Weicong Liu et al. reported that MOF can be used as a potential drug carrier (Zahraa et al., 2019). Cyclodextrin metal-organic framework (CD-MOF) was more suitable for use as a drug nanocarrier. Cyclodextrin metal-organic framework (CD-MOF) is formed by combining cyclodextrin as an organic ligand with potassium ions through coordination bonds. Cyclodextrin (CD) is a green nontoxic and has good biocompatibility (Georgeta et al., 2001; Smaldone et al., 2010; Crini, 2014; Hesler et al., 2020). Besides, CD-MOF has a porous body center structure with a large specific surface area and internal cavity, which is suitable for the loading and transportation of most drug molecules (Li et al., 2017; 2019; Pires et al., 2019; Musumeci et al., 2020). In recent years, with the in-depth study of CD-MOF, more and more researchers focused on drug carriers. Zhang's group (Liu et al., 2017) synthesized CD-MOF nanoparticle modification with cholesterol. The engineered CD-MOF-CHS nanoparticles possessed high DOX absorption capacity and can improve the pharmacokinetics of the drug. Lan's group (Kurek et al., 2011) constructed two β-CD based MOFs, which exhibited a high delivery capacity for anti-cancer drug molecules (Fluorouracil). Compared with the 5-FU/MTX delivery, CD-MOFs showed a slower release rate of 5-FU. At the same time, the cumulative release rates of CD-MOFs were much higher than 5-FU/MTX delivery. These article indicated CD-MOFs favorable potential of being used as effective drug carriers. With the help of nanocarriers, not only can the drug be efficiently delivered to the target site, but also the amount and frequency of dosage can be controlled, thereby preventing toxicities related to therapy (Mura et al., 2013).

In this work, we synthesized γ-CD-MOF, which was confirmed as biocompatible drug carriers by cytotoxicity assays and exhibited a high delivery capacity for antibiotics to the treatment of infections. The antibiotic was primarily encapsulated into the pore structures of γ-CD-MOF, which allowed the sustained release of antibiotics over an extended period of time. The antibiotics that were chosen for this study were some of the most efficient drugs – florfenicol and enrofloxacin. The florfenicol and enrofloxacin were animal special medicine and were known to be highly active against various Gram-positive and Gram-negative bacteria. The γ-CD-MOF was prepared via an ultrasonic method to achieve small-sized particles. Due to the porous structure of γ-CD-MOF, florfenicol and enrofloxacin could be adsorbed into the pores of γ-CD-MOF depending on the antibiotics/γ-CD-MOF ratio. The bactericidal efficacy of antibiotics/γ-CD-MOF was also systematically studied. Notably, antibiotics/γ-CD-MOF showed much superior activity against bacteria than free antibiotics (lower MIC value) and displayed better long-lasting activity (longer antibacterial time). The antibiotics/γ-CD-MOF show nontoxic and biocompatible to mammalian cells both *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials

Potassium hydroxide, γ-cyclodextrin (γ-CD), methanol (analytical reagent) Polyethylene glycol (Mn = 20,000) Poloxamer 408 (F127), Poloxamer181 (L63) were purchased from Kelong Chemical Engineering Company (Chengdu, China). Florfenicol and enrofloxacin were purchased from Shanghai Yuanye Biological Technology Co., Ltd. *E. coli* (ATCC 25922), *S. aureus* (ATCC 43300) were purchased from Shanghai Micro Technology Co., Ltd. Female rabbits were purchased from Chengdu Dashuo Laboratory Animal Co., Ltd.

2.2. Instruments and measurements

Scanning electron microscope (SEM) images of the surface of the fibers were obtained under a scanning electron microscope (JSM-7500F, JEOL, Tokyo, Japan). X-Ray Powder Diffraction (XRD) was collected by PANalytical B.V. Low-pressure gas adsorption experiments were performed on a Quantachrome AUTOSORB-1 automatic volumetric instrument. The UV/Vis and fluorescence spectra results were detected using Varioskan LUX (Thermo, USA). High-performance liquid chromatography (HPLC) spectra results were analyzed by Perkin Elmer Altus.

2.3. Experimental section

2.3.1. Preparation of γ-CD-MOF

KOH (112 mg) and γ-CD (324 mg) were dissolved in purified water (10 mL). And MeOH (6 mL) was added to the solution.

The mixed solution was heated via ultrasound for 1 h until the clear solution was obtained. Then, 128 mg of polyethylene glycol (Mn = 20,000) was added quickly to trigger the rapid deposition of crystalline. After 12 h, white crystals which were appeared at the bottom of the vessel, were isolated and washed with MeOH three times and dried overnight at 50 °C under vacuum.

In order to enhance the stability of γ-CD-MOF, pluronic L63 was encapsulated on the surface of γ-CD-MOF via an impregnation approach. 5 mL pluronic L63 was dissolved in 20 mL methanol and γ-CD-MOF (1 g) was added into this solution. The mixture solution was in reaction for 48 h at room temperature. After the reaction was completed, the precipitate was obtained by filtration and washed with methanol several times. Finally, the product was dried at 50 °C under vacuum for 12 h.

2.3.2. Drug-loaded γ-CD-MOF

The antibiotics selected in this experiment were florfenicol and enrofloxacin. The prepared concentration of antibiotics was 2 mg/mL. Then, γ-CD-MOF (25 mg) was added to the above solution. The volume of the antibiotics solution was 2 mL. After 24 h, the solid was filtered and washed three times with methanol to ensure that the free drug adsorbed on the surface was completely removed. The obtained solid powder was dried at 37 °C.

2.3.3. In vitro drug release studies

Firstly, antibiotic solutions with different concentration gradients were prepared and measured the values of absorbance respectively. Then, it was to establish the standard curve with concentration as the horizontal axis and absorbance as the vertical axis. Secondly, The drug-loaded γ-CD-MOF (250 mg) was placed in a dialysis bag (Mn = 500) and dialysis in 50 mL PBS buffer solutions at 37 °C. At certain time intervals, the absorbance value of the dialysis solution was measured at 334 nm (enrofloxacin) and 266 nm (florfenicol) by UV/Vis spectra. According to the standard curve, the concentration of the drug was calculated. Thus, the number of antibiotics released was determined and the cumulative release curves of the drug can be drawn.

2.3.4. In vitro antibacterial activity

The minimum inhibitory concentrations (MICs) of antibiotics and drug-loaded γ-CD-MOF were determined using a broth microdilution method. Various concentrations of drug-load γ-CD-MOF (drug concentration: 1024, 512, 25.6, 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1, 0.05, 0.025, 0.0125, and 0 μg/mL) were prepared. The bacterial suspension (100 μL) was seeded onto a 96-well plate and 100 μL of drug-load γ-CD-MOF solution was added. Finally, bacterial growth was determined by measuring the OD values of the bacterial suspension.

The antibacterial activity of drug-load γ-CD-MOF was further examined by disk diffusion assay. About 50 μL of microbial suspensions (*S. aureus* and *E. coli*) were spread on MH agar plates. Disks were prepared to contain free antibiotics and drug-load γ-CD-MOF by adding 5 μL of the sample solution. Disks were air-dried before being placed in each agar plate. Agar plates were incubated for 12 h at 37 °C and measured the diameter of the inhibition zone.

2.3.5. Long lasting antibacterial activity

The solution of drug-load γ-CD-MOF and free antibiotics was prepared. The bacterial suspension (100 μL) was seeded onto a 96-well plate and 100 μL of drug-load γ-CD-MOF solution was added. After incubated for 6, 12, 18, and 24 h, the OD value of bacterial suspension was recorded.

2.3.6. In vitro toxicity

L929 cells were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China) and were applied for evaluating the cell viability via the MTT assay. L929 cells were cultivated in a 96-well culture plate in DMEM medium for 24 h. Then, the cells were treated with γ-CD-MOF and drug-loaded γ-CD-MOF varying concentrations of 1–100 μg/mL for 24 h. To determine toxicity, 10ul of MTT solution was added to each well of the plate and incubated for 4 h. The absorbance was measured at 570 nm. The relative cell viability was calculated as the percent ratio of the absorbance of γ-CD-MOF and drug-loaded γ-CD-MOF to control.

2.3.7. In vivo antibacterial activity

The female rabbits were randomly grouped (2 rabbits in each group) and assigned to three treatment groups: saline (control), free antibiotics and drug-loaded γ-CD-MOF. The antibiotics and drug-loaded γ-CD-MOF was administered once at a dose of 0.5 mg/Kg via subcutaneous injection and blood samples were collected from the marginal ear vein at each time point of 5 min, 15 min, 30 min, 45 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, 18 h, 24 h, 48 h. Plasma was separated by centrifugation at 12,000 r/min for 3 min and frozen at −20 °C, and the drug concentration of plasma was measured by high-performance liquid chromatography.

3. Results and discussion

3.1. Morphology and structure of γ-CD-MOF and drug-loaded γ-CD-MOF

The preparation of crystalline, homogeneous, and stable CD-MOFs was important to the drug delivery system. In the present investigation, the γ-cyclodextrin metal-organic framework (γ-CD-MOF) was synthesized via the ultrasonic method. In order to improve the stability of γ-CD-MOF, pluronic L63 was encapsulated on the surface of nanoparticles via an impregnation approach. The scanning electron microscopy analysis was conducted to characterize the morphology and size of the prepared γ-CD-MOF samples, as shown in Figure 1. The pluronic L63-modified γ-CD-MOF had similar morphologies and size distributions to their parent γ-CD-MOF (Figure 1(a,b)). These SEM images showed that the grafting reaction did not affect the molecular structures of γ-CD-MOF. To study the crystallinity of γ-CD-MOF and pluronic L63-

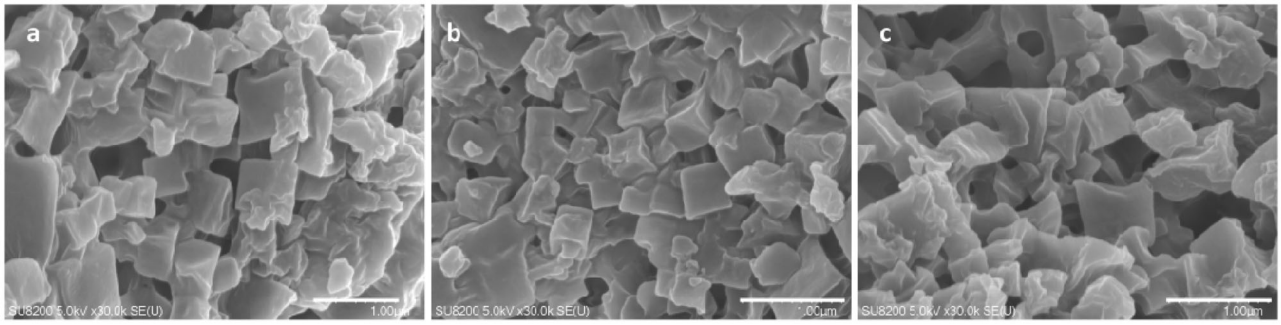

Figure 1. The SEM images of γ-CD-MOF (a), L63-modified γ-CD-MOF (b), enrofloxacin loaded- γ-CD-MOF (c).

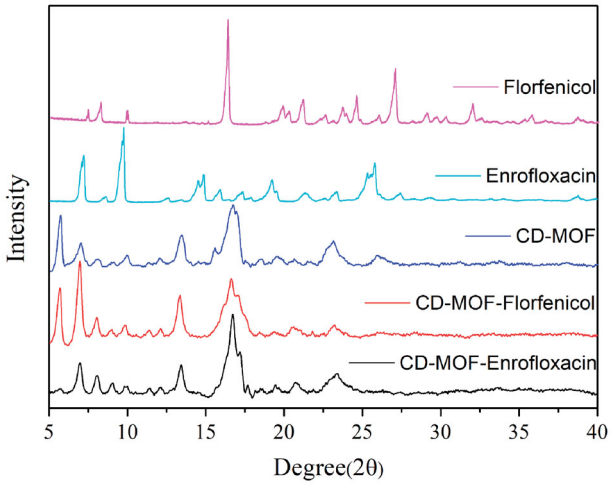

Figure 2. PXRD crystallinity patterns of γ-CD-MOF, drug-loaded γ-CD-MOF and drugs.

modified γ-CD-MOF, we performed X-ray powder diffraction (XRD) analysis. The XRD results (Figure 2) show that the crystalline morphology of γ-CD-MOF was consistent with those reported. It confirmed that these patterns were no significant change and the crystalline structure of the γ-CD-MOF was the same as their modification with pluronic L63. All the results indicated that the crystal structure and morphology of the nanoparticles were the same after modified with the pluronic L63.

The porous structure of CD-MOFs had reported that it can encapsulate the drug Ibuprofen (Li et al., 2017). According to the reported literature, the solvent was an important factor to affect the loading efficiency and the best loading was recorded in methanol and ethanol. Therefore, in this work, we investigated γ-CD-MOF nanocrystals to encapsulate enrofloxacin and florfenicol in ethanol by using the impregnation approach. Based on the standard curve, the γ-CD-MOF particles were able to load antibiotics and the adsorption capacities could reach 45.25 and 54.6 mg/g (Figures S1 and S2). The effect of antibiotics adsorbing into the pore structure of γ-CD-MOF was studied before and after drug incorporation (Figure 3). As expected, the BET surface areas were decreases dramatically after encapsulated drug. The BET surface areas were decreased from 1149.56–480.94 m^2/g. This result further confirmed that the γ-CD-MOF particles can encapsulate the antibiotics efficiently. Furthermore, the crystalline structures of γ-CD-MOF were practically unchanged after drug incorporation. X-ray diffraction patterns were shown in Figure 2. It revealed that enrofloxacin and florfenicol loading γ-CD-MOF was in agreement with the crystallinity of the γ-CD-MOF. The PXRD peaks of enrofloxacin and florfenicol were quite different from γ-CD-MOF. All these results indicated that the enrofloxacin and florfenicol were entrapped into the pore of γ-CD-MOF.

3.2. The antimicrobial activity of drug-loaded γ-CD-MOF

Next, the minimum inhibitory concentrations (MICs) of free antibiotics and drug-loaded γ-CD-MOF was evaluated using a broth microdilution. Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli* were selected as pathogenic bacteria to study the antimicrobial activity. The MIC values were listed in Table 1. The MIC values for free enrofloxacin and enrofloxacin-loaded γ-CD-MOF were estimated to be 0.8 and 0.4 μg/mL, thus implying that enrofloxacin-loaded γ-CD-MOF can inhibit the growth of *S.aureus* more effectively than free enrofloxacin. However, the growth of *S.aureus* was more effectively inhibited when treated with free florfenicol than florfenicol-loaded γ-CD-MOF. The MIC values for free florfenicol and florfenicol-loaded γ-CD-MOF were estimated to be 1.6 and 3.2 μg/mL respectively. In the case of *E.coli*, both enrofloxacin and florfenicol-loaded γ-CD-MOF required higher inhibiting activity than the free drug. The MIC values for enrofloxacin and florfenicol-loaded γ-CD-MOF were 0.05 μg/mL and 0.05 μg/mL. According to the reported lectures (Wu et al., 2013; Zhang et al., 2016; Jia et al., 2017), nanoparticles encapsulated kinds of functional molecules could be uptaken effectively to enhance the therapeutic effect. Thus, we speculate that the drug-loaded γ-CD-MOF could be easily phagocytosed by the cell to reduce the MIC value. To further investigating the inhibiting activity, it was evaluated using a disk diffusion test. For both types of microbes, the inhibition zones were clearly observed around dick containing enrofloxacin and florfenicol-loaded γ-CD-MOF and free drug. The inhibition zone area of drug-loaded γ-CD-MOF for *E.coli* and *S.aureus* was similar to the free drug. It was because that the γ-CD-MOF could increase the solubility of antibiotics (Table S2) and improve the bioavailability. Therefore, it was indicated that drug-loaded γ-CD-MOF could improve the antimicrobial activity compared with free drug.

In order to establish that the drug-loaded γ-CD-MOF could release the antibiotics over a long period of time. Thus we studied the release of antibiotics from drug-loaded γ-CD-

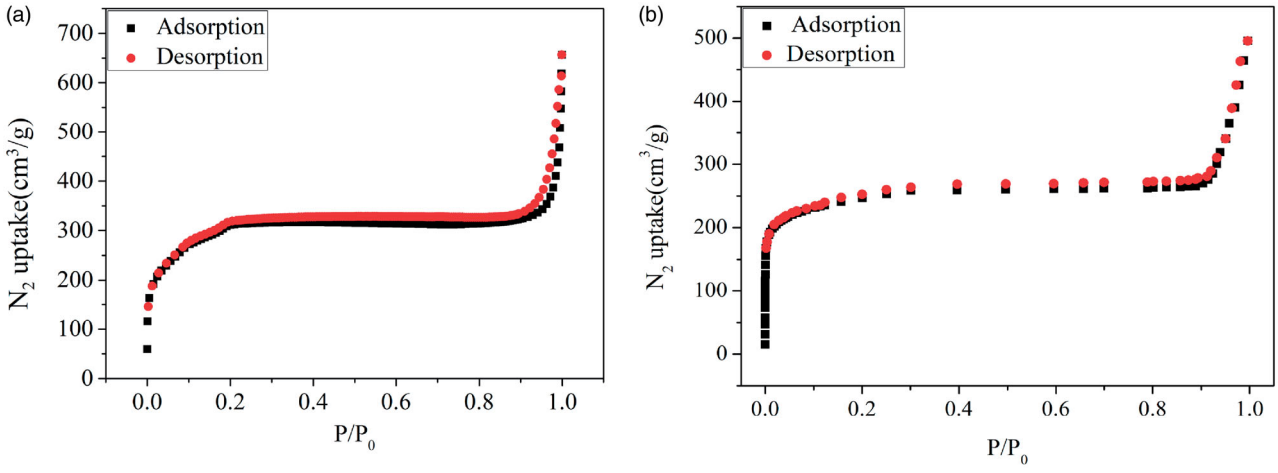

Figure 3. N_2 adsorption isotherms of γ-CD-MOF (a) and drug-loaded γ-CD-MOF (b).

Table 1. Antimicrobial activity of free antibiotics and antibiotics-loaded γ-CD-MOF.

	E. coli		*S. aureus*	
	MIC (μg/mL)	Inhibition zone (cm)	MIC (μg/mL)	Inhibition zone (cm)
Control				
Enrofloxacin	0.2	1.81 ± 0.10	0.8	1.36 ± 0.57
Florfenicol	0.8	1.90 ± 0.05	1.6	1.85 ± 0.05
Enrofloxacin loaded γ-CD-MOF	0.05	1.93 ± 0.05	0.4	1.44 ± 0.76
Florfenicol loaded γ-CD-MOF	0.10	2.02 ± 0.15	1.6	1.96 ± 0.15

Table 2. Safely evaluation of drug-loaded γ-CD-MOF *in vivo* was evaluated by analyzing hematology.

Analytes	Enrofloxacin	γ-CD-MOF	Enrofloxacin loaded γ-CD-MOF	Normal range
WBC ($\times10^9$/L)	7.50 ± 0.14	6.86 ± 0.09	7.10 ± 0.55	3–13.50
LTM ($\times10^9$/L)	5.05 ± 0.07	5.01 ± 0.09	4.97 ± 0.28	1.00–6.80
MON ($\times10^9$/L)	0.52 ± 0.04	0.30 ± 0.02	0.38 ± 0.09	0.08–1.51
NEU ($\times10^9$/L)	1.83 ± 0.03	1.49 ± 0.04	1.70 ± 0.09	0.50–6.60
PLT ($\times10^9$/L)	479 ± 3.60	481 ± 3.79	492 ± 13.00	100–1250
RBC ($\times10^{12}$/L)	5.79 ± 0.11	5.21 ± 0.04	5.48 ± 0.29	3.40–6.50
HGB (g/L)	127 ± 0.58	114 ± 0.58	120 ± 7.23	80–140
MCV (Fl)	68.50 ± 0.53	67.60 ± 0.21	67.60 ± 0.68	60–80
MCH (pg)	21.90 ± 0.35	21.70 ± 0.06	21.70 ± 0.49	19–25
MCHC (g/L)	320 ± 6.08	322 ± 1.15	322 ± 7.50	300–360

MOF *in vitro* at pH 7.4. The antibiotics sustained release was shown in Figure 4. Drug-loaded γ-CD-MOF was shown to release the antibiotics continuously over 4 hours. Florfenicol-loaded γ-CD-MOF showed 79.85% release and 87.50% release of enrofloxacin-loaded γ-CD-MOF was observed after 4 hours. It should also be mentioned that 40% of antibiotics (florfenicol and enrofloxacin) was released within the first 1 h from γ-CD-MOF. And the gradual release of antibiotics was continued over 4 h. The above results indicated that the porous structure of γ-CD-MOF could in controlling the release behavior of the antibiotic.

As the antibiotic was gradually released, and hence drug-loaded γ-CD-MOF was able to display long-lasting antibacterial activity. Herein we tested the antibacterial activity of the released antibiotic collected in nutrient media and measured the OD value of bacterial suspension at certain time intervals (In Table S1). The bacterial inhibition rate was calculated as the percent ratio of the OD value of free antibiotic and drug-loaded CD-MOF to control. If there was any release of antibiotics from CD-MOF, the bacterial growth should as well as a control group. According to the result (Figure 5(a)), the inhibition rate of drug-loaded CD-MOF reached 90% and was much higher than the free drug. It suggested that drug-loaded CD-MOF showed a higher and longer inhibition ability of bacteria. Thus, indicating that these drug-loaded CD-MOF gradually released antibiotics in the solution leading to high bacterial inhibition.

As shown in Figure 5(b), we compared the drug concentration in blood with a different time interval. Within the first 5 min, the concentration of free enrofloxacin was a little higher than that of enrofloxacin-loaded γ-CD-MOF. Meanwhile, with the metabolism and blood circulation, the concentration of free drug was gradually decreased. The solubility of enrofloxacin-loaded γ-CD-MOF was much better than that of enrofloxacin (Table S2). As well as the drug-loaded γ-CD-MOF could release the drug within 4 hours persistently and slowly *in vitro*. The free drug was rapidly cleared from blood circulation in comparison with the same doses of enrofloxacin-loaded γ-CD-MOF. Thus, the concentration of Enrofloxacin-loaded γ-CD-MOF could maintain a high concentration for nearly 2 h. Enrofloxacin-loaded γ-CD-MOF shows higher drug concentration and half-lives than free enrofloxacin. It was indicated that γ-CD-MOF could improve the adsorption and utilization efficiency of enrofloxacin.

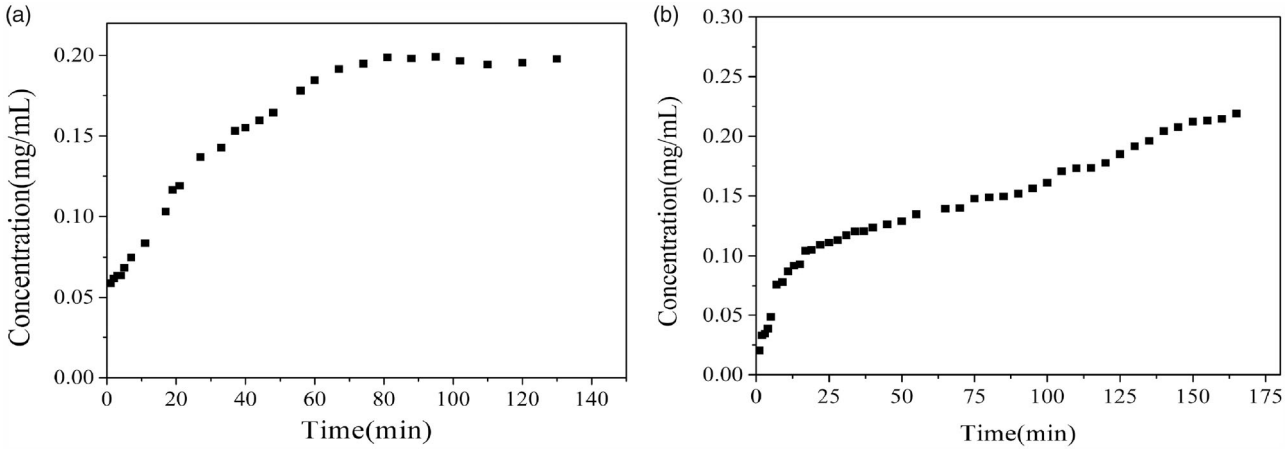

Figure 4. The cumulative release curves of enrofloxacin (a) and florfenicol (b) from γ-CD-MOF. The amount antibiotic concentration in the solution was determined by UV-visible absorption spectroscopy.

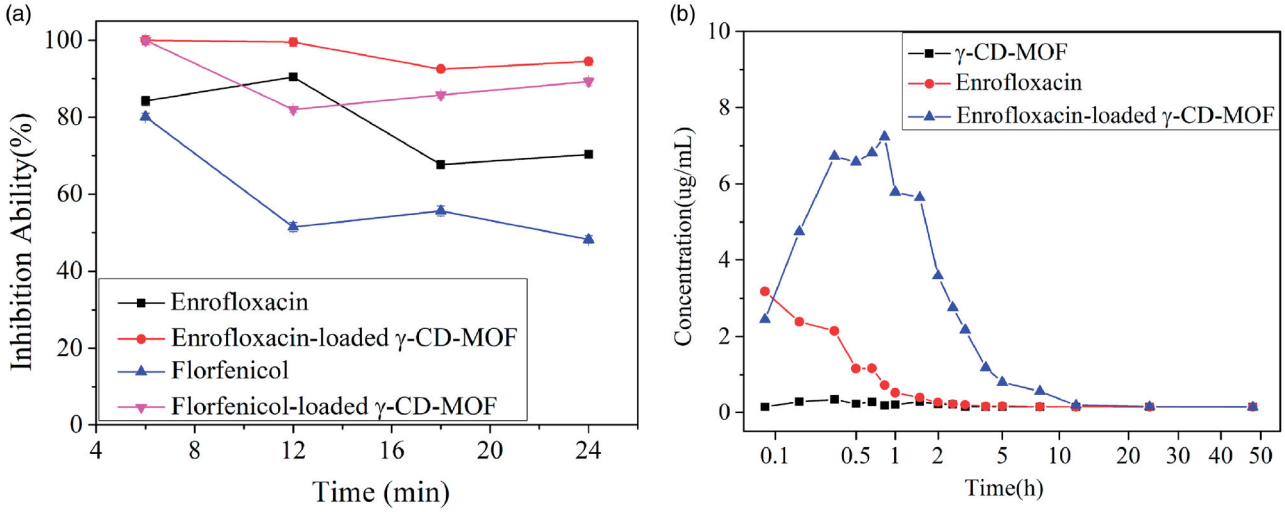

Figure 5. (a) Antibacterial activity of γ-CD-MOF and drug-loaded γ-CD-MOF. (b) Concentration-time profiles of enrofloxacin in plasma of female rats after a single subcutaneous administration of free enrofloxacin and enrofloxacin-loaded γ-CD-MOF (equivalent to 0.5 mg/kg enrofloxacin).

Moreover, the enrofloxacin loaded γ-CD-MOF could gradually release enrofloxacin *in vivo*. Therefore, the γ-CD-MOF as the perfect vehicle could sustain the release of enrofloxacin both *in vitro* and *in vivo* and show longer inhibition ability of bacteria. The dosage of antibiotics can be greatly reduced and also can be achieved good therapeutic effects.

3.3. The biocompatibility of drug-loaded γ-CD-MOF

The biocompatibility of the drug carrier was of critical importance to its success. The cytotoxicity of γ-CD-MOF and enrofloxacin-loaded γ-CD-MOF was measured in L929 cells over 48 h using MTT assays. The DMEM medium group was used as a control group. The relative dose-dependent cell viability percentages were calculated as the rate of the control group. The results were shown in Figure 6. The cell viability also remained high enough and no significant decrease in cell viability was observed with the increase of concentration from 1 to 100 μg/mL. Therefore, the results indicate a high degree of safety with regard to cytotoxicity, even at a maximum concentration of 100 μg/mL.

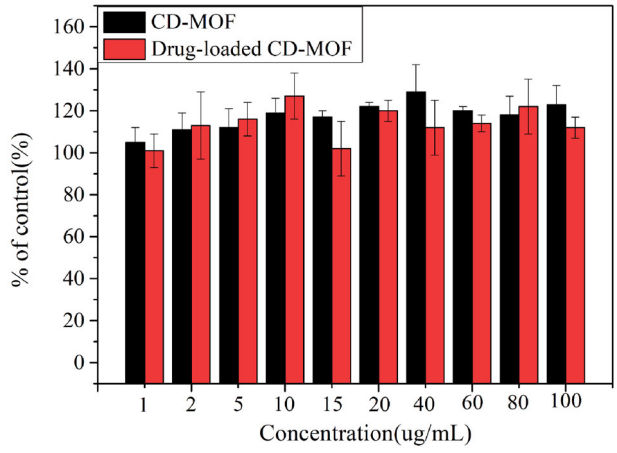

Figure 6. Relative cell viabilities of L929 cells incubated with different concentrations of γ-CD-MOF and enrofloxacin-loaded γ-CD-MOF.

To investigate the toxicity of drug-loaded γ-CD-MOF *in vivo*, healthy mice were administered enrofloxacin-loaded γ-CD-MOF, γ-CD-MOF or enrofloxacin via subcutaneous injection for 7 days. After the experiment, drops of blood were collected for hematological and biochemical analyses, and

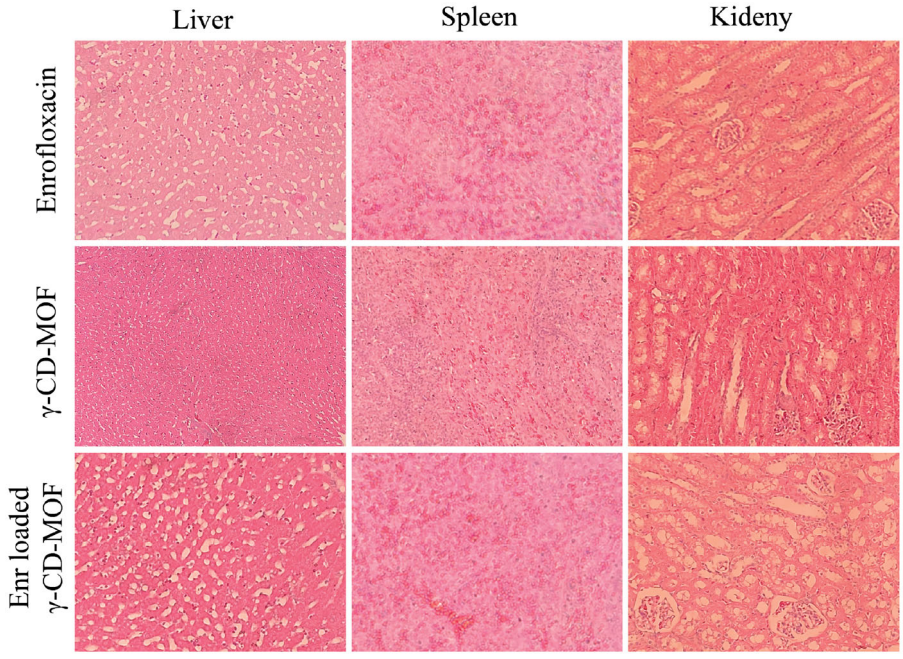

Figure 7. Hematoxylin and eosin (H&E) staining of histological sections (×10) was used to assess the toxicity of drug-loaded γ-CD-MOF toward tissues (liver, spleen and kidney).

tissues from major organs (liver, spleen, and kidney) were collected for histological analysis. There was no remarkable difference in hematological of the treated mice compared with the control group (Table 2). Hematoxylin and eosin (H&E) staining of histological sections showed in Figure 7. The HE staining revealed that the organ and tissue morphogenesis treated with γ-CD-MOF and enrofloxacin-loaded γ-CD-MOF were nearly the same as the control group (treated with enrofloxacin). These results indicated that enrofloxacin-loaded γ-CD-MOF and γ-CD-MOF did not have any obvious toxicity *in vivo*. Thus, γ-CD-MOF exhibited excellent biocompatibility *in vitro* and *in vivo* and was safely used as a drug delivery vehicle.

4. Conclusion

In this study, we synthesized the delivery vehicle named γ-cyclodextrin metal-organic framework (γ-CD-MOF) via the ultrasonic method. The prepared γ-CD-MOF contained high porosity and large specific surface area, which can encapsulate drugs efficiently. The loading capacity on florfenicol and enrofloxacin reached 54.6 mg/g and 45.25 mg/g respectively. Furthermore, the γ-CD-MOF released florfenicol and enrofloxacin over a significantly extended period of time and display better and long-lasting antibacterial activity. The MIC value for florfenicol-loaded and enrofloxacin-loaded CD-MOF in *E. coil* and *S. aureus* were much lower than the free antibiotics. *In vitro* study, the drug-loaded γ-CD-MOF maintained higher and longer inhibition activity than free antibiotics. *In vivo*, drug-loaded γ-CD-MOF also shows no toxicity and perfect biocompatibility toward the animal. In this work, the drug-loaded γ-CD-MOF allowed a sustained release drug, show low MIC value, and display high half-lives. Therefore, γ-CD-MOF could be potentially used to deliver antibiotics and as an excellent vehicle for the therapy against bacteria. The drug-loaded γ-CD-MOF could change oral administration to injection, enrich the dosage forms and improve the bioavailability of antibiotics in the body, to reduce the development of resistance.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was financially supported by National Science Foundation of China [21908183], Science and Technology Training Planning Project of Sichuan Province [2016KZ0007] and Fundamental Research Funds for Central Universities, Southwest Minzu University [2020NYB28].

ORCID

Tao Dai http://orcid.org/0000-0002-6702-888X

References

Anand A, Das P, Nandi SK, Kundu B. (2020). Development of antibiotic loaded mesoporous bioactive glass and its drug release kinetics. Ceram Int 46:5477–83.

Andersson DI, Hughes D. (2011). Persistence of antibiotic resistance in bacterial populations. FEMS Microbiol Rev 35:901–11.

Atkinson I, Anghel EM, Predoana L, et al. (2016). Influence of ZnO addition on the structural, *in vitro* behavior and antimicrobial activity of sol-gel derived CaO-P_2O_5-SiO_2 bioactive glasses. Ceram Int 42: 3033–45.

Brooks BD, Davidoff SN, Grainger DW, Brook AE. (2013). Comparisons of release of several antibiotics from antimicrobial polymer-coated allograft bone void fillers. Int J Biomed Mater Res 1:21–5.

Crini G. (2014). Review: a history of cyclodextrins. Chem Rev 114: 10940–75.

Doan HV, Hamzah HA, Prabhakaran PK, et al. (2019). Hierarchical metal-organic frameworks with macroporosity: synthesis, achievements, and challenges. Nano-Micro Lett 11:281–313.

Georgeta M, Despina V, Carpov A. (2001). Cyclodextrin polymers. Cyclodextrin Polym. J Bioactive Compat Polym 16:315–42.

Hesler M, Schwarz DH, Dähnhardt-Pfeiffer S, et al. (2020). Synthesis and *in vitro* evaluation of cyclodextrin hyaluronic acid conjugates as a new candidate for intestinal drug carrier for steroid hormones. Eur J Pharm Sci 143:105181.

Hilal D, Seda K. (2018). Computational screening of metal-organic frameworks for membrane-based $CO_2/N_2/H_2O$ separations: best materials for flue gas separation. J Phys Chem C, Nanomater Interf 122: 17347–57.

Hou Y, Hu XJ, Tong HY, et al. (2020). Unraveling the relationship of the pore structures between the metal-organic frameworks and their derived carbon materials. Inorg Chem Commun 114:107825.

Jia JL, Liu Q, Yang TY, et al. (2017). Facile fabrication of varisized calcium carbonate microspheres as vaccine adjuvants. J Mater Chem B 5: 1611–23.

Kaur H, Sundriyal S, Pachauri V, Ingebrandt , et al. (2019). Luminescent metal-organic frameworks and their composites: potential future materials for organic light emitting displays. Coord Chem Rev 401: 213077.

Kurek A, Grudniak AM, Kraczkiewicz-Dowjat A, Wolska KI. (2011). New antibacterial therapeutics and strategies. Pol J Microbiol 60:3–12.

Li HY, Lv N, Li X, et al. (2017). Composite CD-MOF nanocrystals-containing microspheres for sustained drug delivery. Nanoscale 9:7454–63.

Li WQ, Xu CF, Li SX, et al. (2019). Cyclodextrin based unimolecular micelles with targeting and biocleavable abilities as chemotherapeutic carrier to overcome drug resistance. Mater Sci Eng C Mater Biol Appl 105:110047.

Lin RB, Xiang SC, Zhou W, Chen BL. (2020). Microporous metal-organic framework materials for gas separation. Chem 6:337–63.

Liu J, Bao TY, Yang XY, et al. (2017). Controllable porosity conversion of metal-organic frameworks composed of natural ingredients for drug delivery. Chem Commun 53:7804–7.

Liu SQ, Fukushima K, Venkataraman S, et al. (2018). Supramolecular nanofibers self-assembled from cationic small molecules derived from repurposed poly(ethylene teraphthalate) for antibiotic delivery. Nanomed: Nanotechnol, Biol, Med 14:165–72.

Liu WC, Zhong YY, Wang XX, et al. (2020). A porous Cu(II)-based metal-organic framework carrier for pH-controlled anticancer drug delivery. Inorg Chem Commun 111:107675.

Mura S, Nicolas J, Couvreur P. (2013). Stimuli-responsive nanocarriers for drug delivery. Nat Mater 12:991–1003.

Musumeci T, Bonaccorso A, De Gaetano F, et al. (2020). A physico-chemical study on amphiphilic cyclodextrin/liposomes nanoassemblies with drug carrier potential. J Liposome Res 30:407–10.

Nathan W, Ockwig OD-F, Michael O, Yaghi OM. (2005). Reticular chemistry: occurrence and taxonomy of nets and grammar for the design of frameworks. Acc Chem Res 38:176–82.

Pires FQ, da Silva JKR, Sa-Barreto LL, et al. (2019). Lipid nanoparticles as carriers of cyclodextrin inclusion complexes: a promising approach for cutaneous delivery of a volatile essential oil. Coll Surf B Biointerf 182: 110382.

Radovic-Moreno AF, Lu TK, Puscasu VA, et al. (2012). Surface charge-switching polymeric nanoparticles for bacterial cell wall-targeted delivery of antibiotics. ACS Nano 6:4279–87.

Smaldone RA, Forgan RS, Furukawa H, et al. (2010). Metal-organic frameworks from edible natural products. Angew Chem Int Ed Engl 49: 8630–4.

Tian YF, Chen GH, Guo LH, et al. (2015). Methodology studies on detection of aminoglycoside residues. Food Anal Meth 8:1842–57.

Wu CT, Fan W, Chang J. (2013). Functional mesoporous bioactive glass nanospheres: synthesis, high loading efficiency, controllable delivery of doxorubicin and inhibitory effect on bone cancer cells. J Mater Chem B 1:2710–8.

Xiong MH, Bao Y, Yang XZ, et al. (2014). Delivery of antibiotics with polymeric particles. Adv Drug Deliv Rev 78:63–76.

Yang WH, Guo H, Yue LG, et al. (2020). Metal-organic frameworks derived MMoSx (M = Ni, Co and Ni/Co) composites as electrode materials for supercapacitor. J Alloys Compd 834:154118.

Zahraa H, Nathalie K, Lizette A, et al. (2019). Cyclodextrin-membrane interaction in drug delivery and membrane structure maintenance. Int J Pharm 564:59–76.

Zhang Y, Wang F, Ju EG, et al. (2016). Metal-organic-framework-based vaccine platforms for enhanced systemic immune and memory response. Adv Funct Mater 26:6454–61.