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Fabrication of a hyaluronic acid conjugated metal organic framework for targeted drug delivery and magnetic resonance imaging

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Since metal organic frameworks (MOF) have exhibited fascinating potential in biomedical applications, it is worthwhile to construct a MOF-based multifunctional drug delivery system. In the present study, the anticancer drug doxorubicin (DOX) was loaded into zeolitic imidazolate framework-8 (ZIF-8) via a one-pot process. The formed DOX@ZIF-8 was then coated with polydopamine, successively chelated with Fe³⁺ and conjugated with hyaluronic acid (HA), finally resulting in a multifunctional ZIF-8 nanocarrier. The characterization results confirmed the successful formation of the hybrid nanocarrier. pH-responsive drug release of DOX was observed due to the innate pH-dependent stability of ZIF-8. Importantly, the flow cytometry and confocal laser scanning microscope results both verified the targeting ability of DOX@ZIF-HA toward prostate cancer PC-3 cells. The improved therapeutic efficacy of DOX@ZIF-HA when compared to the inhibited group was also demonstrated. Furthermore, the chelation of Fe³⁺ by PDA makes the prepared DOX@ZIF-HA a good contrast agent for magnetic resonance (MR) imaging. Hence, we hope the constructed ZIF-8 based multifunctional nanocarrier could be a candidate for cancer theranostics.

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1. Introduction

As one of the most frequently used approaches for combating cancer, chemotherapy has always suffered some unbearable drawbacks, including non-specific toxicity toward normal cells, premature degradation in circulation and insufficient therapeutic efficacy.¹ To address those problems, various nanocarriers, such as liposomes,² polymer micelles,³ graphene⁴ and mesoporous silica nanoparticles,⁵ have been widely developed for packaging and delivering chemotherapeutic drugs. Nanocarriers can not only protect the drug from rapid clearance, but can also improve its accumulation at the tumor site through the enhanced permeability and retention (EPR) effect.⁶ However, currently explored nanocarriers still have a few shortcomings to conquer. For instance, the stability of organic nanocarriers is poor and they can only offer single functionality,⁷ while debates about the biocompatibility and biodegradability of inorganic nanocarriers have never stopped.⁸ The construction of organic/inorganic hybrid nanocarriers could adequately integrate the functionality of inorganic nanomaterials with the biocompatibility of organic nanomaterials.⁹ Unfortunately, simple hybridization still fails to change their innate physiochemical

properties. As a consequence, the development of novel nanocarriers with a natural organic/inorganic constitution may strongly promote the translation process of nanomedicine.

Metal organic frameworks (MOFs) are highly porous materials composed of metal ions and organic linkers.¹⁰ In particular, nano-scale MOFs have drawn extensive attention owing to their high surface area, tunable shapes and pore sizes, and controllable surface functionalities.¹¹ Built from zinc ions and imidazole units, zeolitic imidazolate framework-8 (ZIF-8) is one of the most widely investigated subclasses of MOFs for biomedical applications.¹² It is well known that ZIF-8 can decompose under lower pH conditions,¹³ thus making it a good candidate for a pH-responsive nanocarrier for drug delivery. For instance, Wang's group¹⁴ developed a facile two-step method to fabricate green fluorescent carbon nanodots@ZIF-8 with adjustable size and fluorescence intensity, then the prepared hybrid ZIF-8 was loaded with 5-fluorouracil and a pH-responsive drug release behavior was demonstrated. In another study, He *et al.*¹⁵ prepared CuS-encapsulated ZIF-8 and then the anti-cancer drug doxorubicin (DOX) was loaded into the hybrid ZIF-8 nanoparticles for combined therapy. However, the drug loading process in those studies was mostly accomplished in two steps: the formation of ZIF-8 and the absorption of the drug. This approach always suffers from low loading efficacy and poorly controlled release of the molecules. Very recently, Zou and co-workers¹⁶ proposed a one-pot synthetic procedure for the preparation of targeted molecule-encapsulated ZIF-8 in the

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2.5. Characterization

The morphology of DOX@ZIF-8 before and after functionalization was observed using JEOL-2100 transmission electron microscopy (TEM) at an operating voltage of 200 kV. Field-emission scanning electron microscopy (FESEM) was performed on a Hitachi S-4800 (Japan). An X-ray diffraction (XRD) pattern was produced on a Rigaku D/MAX-2550 PC diffractometer using monochromic Cu-K α radiation operated at 40 kV and 80 mA in a 2θ range of 10–50°. The size distribution of DOX@ZIF-HA in different media was measured by dynamic light scattering (DLS) on a BI-200SM multiangle dynamic/static laser scattering instrument (Brookhaven, U.S.). Zeta potential measurements were performed on Malvern Zetasizer Nano ZS apparatus. A Fourier transform infrared (FTIR) spectrum was recorded using a Nicolet 6700 spectrometer.

2.6. Drug release behavior

Prior to the drug release experiment, the drug loading efficacy was determined. Briefly, a certain amount of DOX@ZIF-HA was incubated in diluted hydrochloric acid solution overnight and the absorption value of the supernatant solution at 480 nm was measured using a UV-vis spectrophotometer.

To investigate the drug release behavior, 5 mg DOX@ZIF-HA was dispersed in 1 mL buffer solution with different pH values (pH 5.0 and 7.4), and sealed in a dialysis bag (MWCO ~ 3500 Da). The dialysis bag was immersed in the corresponding buffer solutions and shaken at 37 °C. The release medium was taken out for UV-vis spectrophotometer measurements and fresh medium was supplied at each time point.

2.7. MR imaging ability

The obtained DOX@ZIF-HA was dispersed in DI water at different Fe concentrations. The T_1 relaxometry of those dispersions was measured using a 0.5-T NMI20-Analyst NMR Analyzing and Imaging system (Shanghai Niumag Corporation, China). Then the T_1 relaxivity was calculated using a linear fit of the inverse T_1 ($1/T_1$) value as a function of Fe concentration. Correspondingly, the T_1 -weighted images of those dispersions were also recorded.

2.8. Cell culture

The human prostate carcinoma cell line (PC-3) was provided by American Type Tissue Collection (ATTC, Rockville, MD). The mouse fibroblast cell line (L929) was purchased from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). PC-3 cells and L929 cells were grown in RPMI-1640 medium and DMEM medium, respectively, supplemented with 10% FBS and 1% penicillin–streptomycin (v/v) and cultured in a humidified atmosphere with 5% CO₂ at 37 °C.

2.9. *In vitro* cellular uptake

The cellular uptake of DOX-incorporated nanoparticles was first investigated using flow cytometry (FCM). PC-3 cells were detached by trypsin and seeded in a six-well plate at a density of 2×10^5 cells per well. After the cells were completely adhered,

the medium was discarded and fresh medium containing free DOX and DOX@ZIF-HA was added. The cells were cultured for another 2 h. Afterward, the cells were trypsinized, washed with PBS several times, and filtered with 400-mesh sieves. The cells were then suspended in PBS for FCM analysis.

The cellular uptake of DOX-incorporated nanoparticles was also inspected by confocal laser scanning microscopy (CLSM). Typically, 2×10^5 PC-3 cells or L929 cells were seeded in a glass-bottom culture dish and incubated overnight. Then the cells were co-cultured with free DOX and DOX@ZIF-HA at a DOX concentration of $5 \mu\text{g mL}^{-1}$ for 2 h. Afterwards, the medium was removed and the cells were washed with PBS several times. The cells were stained with 4% paraformaldehyde for 15 min. Finally, the cells were directly observed using CLSM equipped with an oil lens.

For a comparative experiment, PC-3 cells or L929 cells were pre-treated with 10 mg mL^{-1} HA solution for 4 h. The other procedures were performed in the same way.

2.10. *In vitro* therapeutic efficacy

The therapeutic efficacy of DOX-incorporated carriers against PC-3 cells and L929 cells was evaluated using a standard CCK-8 assay. In brief, PC-3 cells or L929 cells were seeded into a 96-well plate at a density of 10^4 cells per well. After being cultured for 24 h, the cells were treated with free DOX, DOX@ZIF-8 and DOX@ZIF-HA at various concentrations (DOX concentrations of 0.5, 1, 2 and $4 \mu\text{g mL}^{-1}$) and maintained for another 24 h. Then the cells were washed with PBS and cultured with CCK-8 working solution for 2 h. The absorbance at 450 nm of each sample was measured using a microplate reader. The cell viability was calculated by the value of the control group divided by the values of the samples. Four parallel experiments were conducted for each group.

To qualitatively observe the therapeutic efficacy, PC-3 cells were cultured and treated using the same aforementioned procedure. Then the cells were slightly rinsed with PBS and stained with Calcein-AM for 15 min. The staining solution was discarded and the cells were imaged using inverted fluorescence microscopy.

2.11. Statistical analysis

All values were reported as mean \pm standard deviation. A one-way analysis of variance (ANOVA) statistical method was conducted to assess the significance of the experimental data. $*p < 0.05$ and $**p < 0.01$ were considered as statistically significant.

3. Results and discussion

3.1. Synthesis and characterization of DOX@ZIF-HA

The preparation of DOX@ZIF-HA is schematically illustrated in Fig. 1. DOX was incorporated into the MOF *via* a one-pot reaction. Initially, DOX would interact with a Zn ion to form a coordination polymer, then the metal organic framework could rapidly form upon the addition of an organic linker, 2-methylimidazole, on the basis of a previously reported theory.¹⁶ Subsequently, the surface of DOX@ZIF-8 was modified with



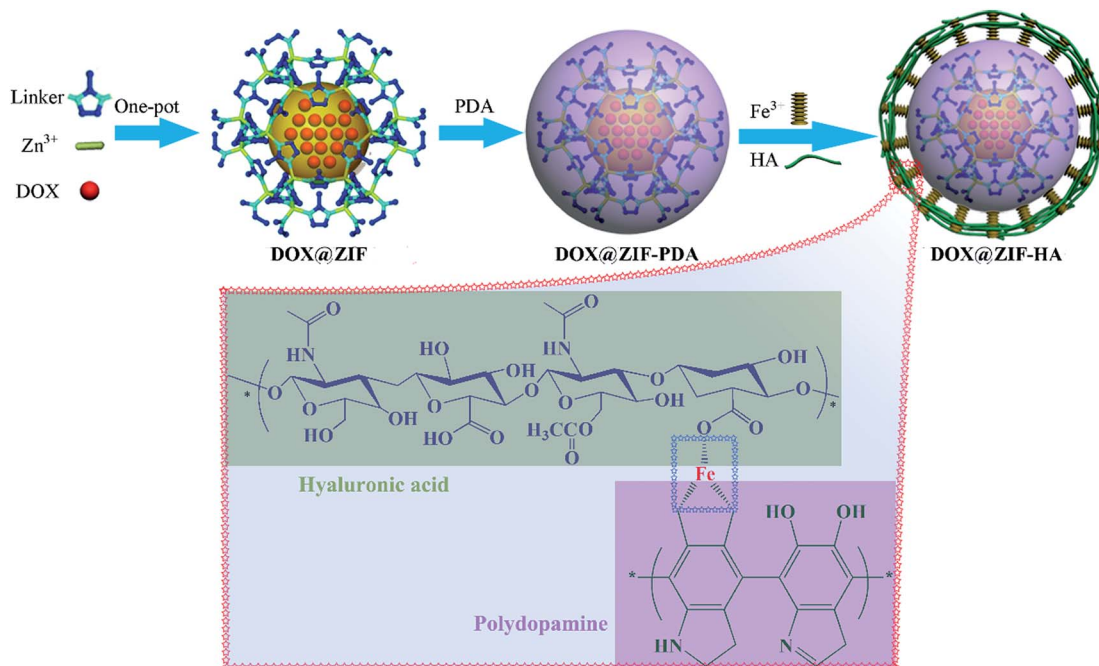


Fig. 1 A schematic diagram for the preparation of DOX@ZIF-HA and the Fe³⁺-mediated coordination interaction between HA and PDA.

PDA, onto which an Fe ion was chelated and HA was conjugated to obtain DOX@ZIF-HA. The prepared MOF, before and after functionalization, was explored by TEM. As presented in Fig. 2B, we successfully fabricated nano-scale DOX@ZIF-8, which clearly displayed a spherical appearance with a diameter of around 150 nm. In contrast, the bare ZIF-8 nanoparticles without DOX loading showed a typical hexagonal structure (Fig. 2A). After functionalization, the TEM images suggest that a more rough surface could be observed (Fig. 2C and D), whereas no

significant change was observed in the morphology of the obtained nanoparticles when compared to pristine DOX@ZIF-8. To further confirm this, an FESEM image of DOX@ZIF-HA is presented in Fig. 2E. It can be easily seen that DOX@ZIF-HA still maintained a round shape and no apparent particle agglomeration occurred, which is beneficial for its application in drug delivery. The crystal phase was also monitored by XRD. As shown in Fig. 2F, pristine DOX@ZIF-8 exhibited well-defined diffraction peaks that correspond to the high crystallinity of

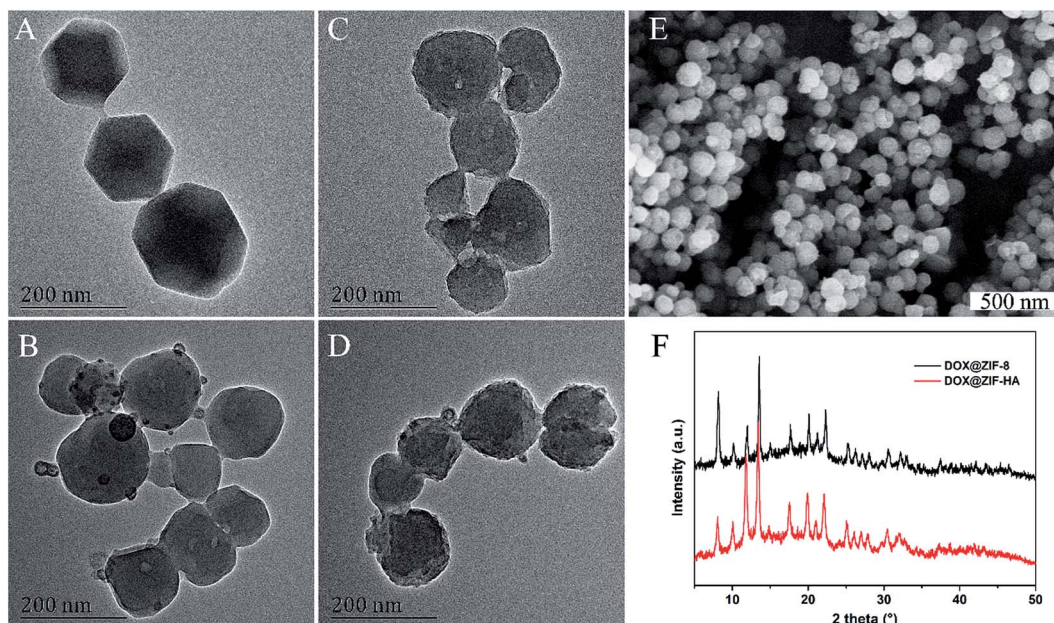


Fig. 2 TEM images of (A) pure ZIF-8, (B) DOX@ZIF-8, (C) DOX@ZIF-PDA and (D) DOX@ZIF-HA nanoparticles. (E) An FESEM image of the prepared DOX@ZIF-HA. (F) The XRD results of the prepared nanoparticles.



ZIF-8.²⁶ Those characteristic peaks were still retained after functionalization was completed, however, a weak broad peak emerged around 20°, which could be assigned to the organic shell.²⁷

Since a good colloidal stability of nanocarriers is a prerequisite for their application, we next inspected the colloidal stability of the as-synthesized DOX@ZIF-HA in different media. DLS was applied to measure the size distribution of those dispersions (Fig. 3A). It was found that the size distribution of DOX@ZIF-HA in PBS and cell culture medium was similar to that in DI water, somewhat reflecting that no agglomeration happened even when DOX@ZIF-HA was dispersed in a solution with a high ionic strength. It was also noticed that the size of DOX@ZIF-HA was slightly increased in FBS, which might be ascribed to the nonspecific adsorbed protein on its surface.²⁸ Meanwhile, the dispersions were stored under ambient conditions for 12 h, and the obtained photograph indicated that no large particles appeared in the dispersions (Fig. 3B), further confirming the colloidal stability of DOX@ZIF-HA in different media.

Zeta potential measurements were performed to detect the surface charge of the prepared nanoparticles. Fig. 4A demonstrates that the as-synthesized DOX@ZIF-8 displayed a positively-charged surface with a zeta potential of 27.1 mV, while PDA modification gives the hybrid nanoparticles a negatively-charged surface owing to the abundant phenolic groups of PDA.²⁹ After the attachment of acid mucopolysaccharide HA,³⁰ the obtained DOX@ZIF-HA still maintained a highly negative potential with a value of -30.2 mV. Logically, the higher negative potential could ensure good colloidal stability of the nanomaterials under physiological conditions,³¹ resulting in good colloidal stability of DOX@ZIF-HA as illustrated above. Afterwards, the chemical composition of the prepared nanoparticles was also investigated using FTIR. As depicted in Fig. 4B, the spectrum of pure ZIF-8 appeared to be typical, and the 1574 and 1145 cm⁻¹ bands belong to C=N stretching vibrations. Moreover, the strong peak at 1077 cm⁻¹, assigned to the C-N stretching of the imidazole units, was observed in both pure ZIF-8 and the other nanoformulations.³² Obviously, the bands in the range of 2800–3200 cm⁻¹ resulted from C-H vibrations in the backbone ring structure.³³ A new peak at

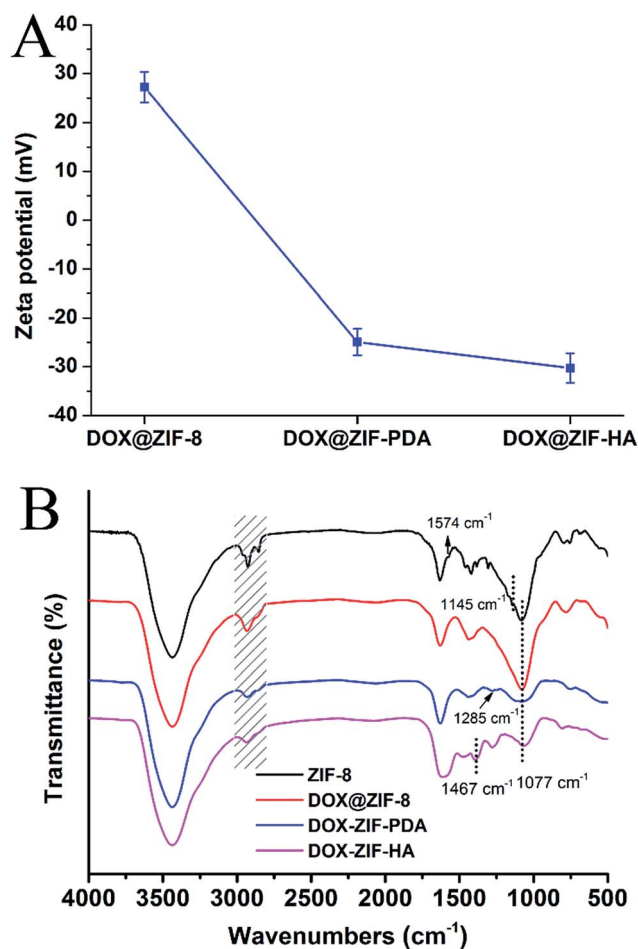


Fig. 4 (A) Surface zeta potential and (B) FTIR spectra of the prepared nanoparticles in different stages.

1285 cm⁻¹, corresponding to the C–O vibration from the phenolic hydroxyl group, was clearly observed after the modification with PDA,³⁴ and the sharp peak at 1077 cm⁻¹ was weakened and broadened at the same time. Besides, the typical absorption peaks of the amide bond were also detected in the spectrum of DOX@ZIF-HA because of the *N*-acetylglucosamine of HA. Specifically, three adjacent peaks at 1610, 1476 and

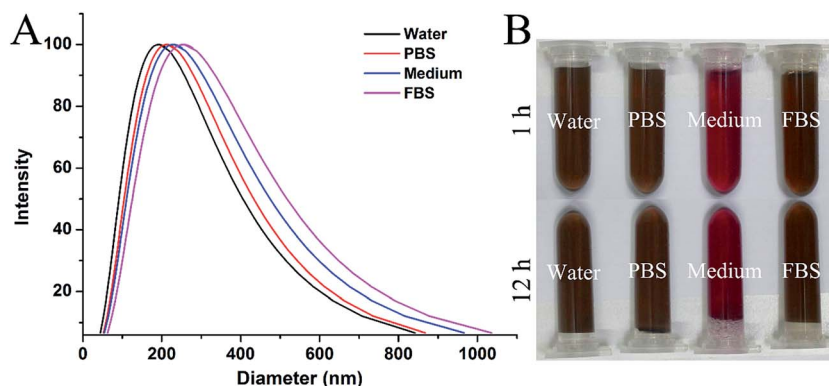


Fig. 3 (A) The size distribution of DOX@ZIF-HA dispersed in different media, as measured by DLS. (B) Photographs of different DOX@ZIF-HA dispersions stored under ambient conditions for 1 h and 12 h.



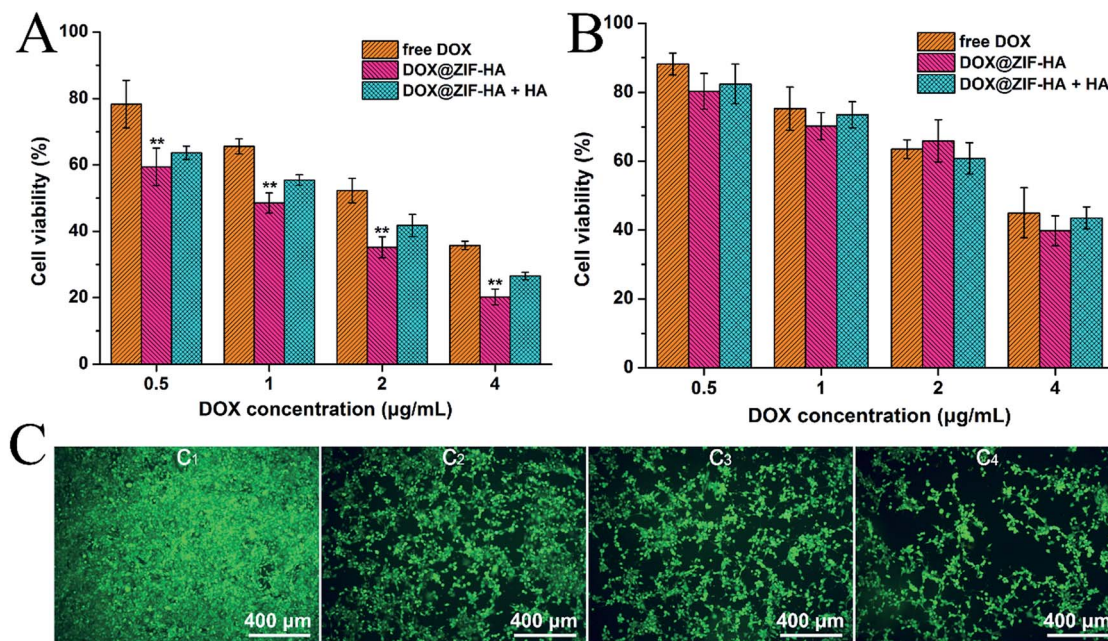


Fig. 8 The CCK-8 assay of (A) PC-3 cells and (B) L929 cells, treated with free DOX and DOX@ZIF-HA at different DOX concentrations for 24 h. (C) Live cell staining (Calcein-AM) of PC-3 cells treated with (C₁) culture medium, (C₂) free DOX, (C₃) DOX@ZIF-HA plus free HA and (C₄) DOX@ZIF-HA, at a DOX concentration of 4 $\mu\text{g mL}^{-1}$.

concentration. Not surprisingly, the inhibition of free HA exerted negligible influence on the therapeutic efficacy of DOX@ZIF-HA. Specifically, at a DOX concentration of 4 $\mu\text{g mL}^{-1}$, the viability for free DOX, DOX@ZIF-HA and DOX@ZIF-HA plus free HA treated L929 cells was 45.04%, 39.84% and 43.56%, respectively. It could be expected that the lower toxicity of DOX@ZIF-HA against L929 would alleviate its side effects toward normal organs, somewhat highlighting the intrinsic merit of actively targeted nanocarriers. Therefore, the above results suggest that the prepared DOX@ZIF-HA could be efficiently applied as an actively targeted nanocarrier to improve chemotherapeutic efficacy.

3.5. *In vitro* MR imaging ability

Apart from mediating the conjugation of HA, the chelation of Fe^{3+} by PDA could also be used as a T_1 contrast agent.^{44,45} So the MR imaging ability of DOX@ZIF-HA was evaluated *in vitro*. As expected, the longitudinal relaxation time (T_1) decreased correlatively with increased Fe concentration (Fig. 9). The r_1 value was calculated to be 5.57 $\text{mM}^{-1} \text{s}^{-1}$, which is higher than a clinically used MRI contrast agent Gd-DTPA. Correspondingly, the MR images of DOX@ZIF-HA dispersions became brighter with the increase of Fe concentration (inset photos in Fig. 9), implying their favorable MR imaging ability. Thus, the DOX@ZIF-HA could also act as an excellent contrast agent for MR imaging.

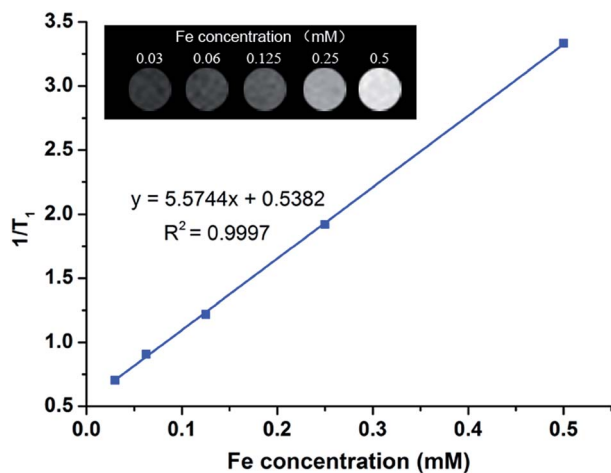
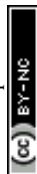


Fig. 9 The *in vitro* MR imaging ability of DOX@ZIF-HA. The linear fitting of $1/T_1$ as a function of Fe concentration. The inset pictures represent the corresponding T_1 -weighted MR images of DOX@ZIF-HA at different Fe concentrations.

4. Conclusion

In summary, DOX-doped MOF nanoparticles were prepared *via* a one-pot reaction and successively anchored with Fe^{3+} and HA for simultaneous targeted drug delivery and MR imaging. The successful construction of DOX@ZIF-HA was confirmed by a series of physicochemical characterization techniques. The incorporated DOX could be released from the nanocarrier in a sustained and pH-sensitive manner. The inhibition experiment also demonstrated that the targeting ability of DOX@ZIF-8-HA toward CD44 overexpressed PC-3 cells could efficiently improve its intracellular uptake and further enhance the *in vitro* chemotherapeutic efficacy as compared to free DOX. Moreover, the chelation of Fe^{3+} endowed DOX@ZIF-HA with a favorable contrast ability for MR imaging. Overall, the developed DOX@ZIF-HA could be applied as a potential theranostic agent for chemotherapy of CD44 overexpressed PC-3 cells and MR imaging.



Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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