A carbon dot-based fluorescent nanoprobe for the associated detection of iron ions and the determination of the fluctuation of ascorbic acid induced by hypoxia in cells and in vivo†

Yan Huang,†a,b Na He,†b,c Qi Kang,*a Dazhong Shen,‡b,d Xiaoyan Wang,†b,c Yunqing Wangb and Lingxin Chen†b,c,d

Maintaining the redox balance of biological systems is a key point to maintain a healthy physiological environment. Excessive iron ions (Fe³⁺) can cause apoptosis, tissue damage and death. Fortunately, ascorbic acid (AA) as a reducing agent has been evaluated for the reduction of Fe³⁺. Moreover, AA plays an important role in relieving hypoxia-induced oxidative stress. Therefore, the real-time imaging of the Fe³⁺ and AA fluctuations is important for understanding their biofunctions in cells and in vivo. In this work, we developed a fluorescent nanoprobe carbon dot-desferrioxamine B (CD-DB) by the conjugate connection of CDs and desferrioxamine B (a complexing agent for Fe³⁺) for the associated detection of Fe³⁺ and AA. CD-DB exhibited excellent sensitivity and selectivity for the detection of Fe³⁺ and AA. The nanoprobe CDs-DB@Fe obtained by the reaction of CD-DB and Fe³⁺ was suitable for tracing the dynamic changes of AA in cells and in vivo. Therefore, CDs-DB@Fe was used for monitoring the fluctuation of AA in hypoxic cell models, hypoxic zebrafish models and liver ischemia mice models. These results exhibited the decrease in AA under hypoxic conditions because AA was consumed to neutralize free radicals and relieve hypoxia-induced oxidative stress damage. The ideal biocompatibility and low toxicity make our nanoprobe a potential candidate for the research of the physiological effects of AA in vivo.

1. Introduction

Iron ions (Fe³⁺) are one of the most important trace elements in vivo and play significant roles in physiological processes, such as oxygen uptake, oxygen metabolism and electronic transfer. Many structural units in the form of an iron complex involve the process of transporting and exchanging oxygen, and several enzymes contain Fe³⁺ as a part of the catalytic sites. Excessive Fe³⁺ causes many diseases, including cancer, organ dysfunction, organ failure, tissue damage and even death. Fortunately, ascorbic acid (AA), also known as vitamin C, can rapidly reduce Fe³⁺ to Fe²⁺ to mitigate the damage of Fe³⁺. AA is often used in health care supplements as an antioxidant. As is well-known, AA is a highly active reducing agent, and it can effectively eliminate free radicals and relieve oxidative stress. Besides, it is a vital vitamin in the diet of humans and has been used for the prevention and treatment of common cold, mental illness, cancer and so on. So far, there are some methods to detect AA and research its biological functions; these include high-performance liquid chromatography (HPLC)/UV spectroscopy, 13C nuclear magnetic resonance spectroscopy [13C NMR]/HPLC and liquid chromatography/mass spectrometry (LC/MS). However, these technologies are not suitable for studying the biological activity of AA due to the destruction of the sample and the irreversible damage. In addition, the highly active AA can be easily destroyed and oxidized by air during the process of sample treatment. Therefore, these technologies cannot utilize the real-time and accurate detection of AA in living cells and in vivo. The fluorescence detection technology using fluorescent nanoprobes exhibits various advantages for the detection of intracellular reactive species, which include greater sensitivity, excellent selectivity, more convenience, less invasive-
ness, and real-time imaging. Therefore, the development of an effective nanoprobe that has high sensitivity and selectivity for the detection and imaging of excessive Fe^{3+} and AA is necessary.

The development of fluorescent nanomaterials has been very quick and has been applied to the field of analysis and medicine, including quantum dots (QDs), carbon dots (CDs), and precious metal nanoclusters. Among them, CDs have attracted researchers’ extensive attention due to their stable photoluminescence, high quantum yield, low toxicity and suitable biocompatibility. Hence, CDs have an excellent potential for the diagnosis and treatment of diseases. Actually, CDs have been widely applied in bioimaging and the design of sensors for the detection of pH, ions (such as Hg^{2+}, Cu^{2+}, Ag^{+}, Fe^{3+}, and PO_{4}^{3−}) and molecular substances (for instance, DNA, nitrite, glucose, and biotin) by monitoring the change in the fluorescence intensity. Recently, some nanoprobe based on CDs, graphene quantum dots (GQDs), gold nanoclusters (AuNCs) and graphitic carbon nitride nanosheets (g-C_{3}N_{4} NNs) have been reported for the detection of Fe^{3+} and AA. These detection methods have high quantum yields, high sensitivity and excellent anti-salt ability. However, some questions also exist in the previous studies: (1) there is a lack of a ligand for fixing Fe^{3+}, which produces non-ideal selectivity; (2) only few of them are applied in actual biological samples (in living cells and in vivo); (3) due to the lack of applications in clinical models, no related nanoprobe has been applied in clinical diagnosis. These problems have hindered the progress of the application of such nanoprobe. In addition, the comparison of such recently reported nanoprobe is shown in Table S1.†

The inner filter effect (IFE), an effective mechanism for fluorescence analysis, was used for the design of the fluorescent nanoprobe. This mechanism has high sensitivity because the changes in the absorption of the nanoprobe can exponentially transform to fluorescence intensity. In this study, we revised a previous synthetic method for the simple one-pot synthesis of CDs, and the CDs were quenched by Fe^{3+} due to IFE. Desferrioxamine B, a complexing agent for Fe^{3+}, conjugated to the surface of CDs to obtain our nanoprobe carbon dot-desferrioxamine B (CD-DB). Then, Fe^{3+} could be reduced to Fe^{2+} by AA, leading to the restoration of fluorescence. Therefore, CD-DB could be used for associatively detecting Fe^{3+} and AA. As a potential nanoprobe, CD-DB exhibited outstanding sensitivity and selectivity for the detection of Fe^{3+} and AA in vitro. The bioassays demonstrated that CD-DB could associatively detect Fe^{3+} and AA in living cells. Furthermore, CDs-DB@Fe (CD-DB combined with Fe^{3+}) was successfully applied to monitor the fluctuation of AA in hypoxic cell models, hypoxic zebrafish models and liver ischemia-induced hypoxic mice model. To the best of our knowledge, this is the first time that a CD-based nanoprobe is applied to analyze the changes in AA in a hypoxic biological sample. Western blot and TUNEL assays were also performed to demonstrate these results. Overall, we hope that our nanoprobe can be applied to clarify the redox dynamic process of AA in biological systems and even in clinical diagnosis.

2. Experimental

2.1 Synthetic procedures of nanoprobe

2.1.1 Synthesis of CDs. Citric acid (5 g, 46.9 mmol) was added to a crucible, and the crucible was heated from room temperature to 300 °C by a muffle furnace for 2 h. Then, a fluffy black solid was obtained, and the crucible was cooled to room temperature. The product, which was a black solid, was combined with 25 mL water and a brown dispersion appeared. This mixture was placed in a dialysis bag (cutoff M_{r}: 1.0 kDa) and dialyzed against water for 2 days to remove small molecules. Finally, CDs were dried in vacuo to give a dark brown solid.

2.1.2. Synthesis of CDs-DB. The CDs (10 mg) was dissolved in 10 mL ultra-pure water with the solution under Ar gas flow to ensure the removal of oxygen. Then, EDC (11.5 mg, 0.06 mmol) and NHS (6.9 mg, 0.06 mmol) were added to the abovementioned solution and stirred for 30 min at room temperature. Desferrioxamine B (10 mg, 0.018 mmol) was added to the abovementioned solution and stirred for 24 h at room temperature under dark conditions. The final solution was then dialyzed by a dialysis bag (cutoff M_{r}: 1.0 kDa) against water for 3 days to remove the unreacted molecular precursors. Finally, the water in the dialyze was lyophilized and CD-DB was obtained as a brown powder.

2.2 Cell staining procedures

HepG2 cells were seeded in a flat-bottom 6-well plate with glass coverslips in 2 mL culture medium. After overnight incubation, the cells were treated with CDs-DB@Fe for 1 h at 37 °C. Then, the treated cells were cultivated with AA (1 mM) for 1 hour. The cells were washed by PBS buffer for three times. Subsequently, the cells were imaged under a laser scanning confocal microscope. Cells were incubated with pure CDs-DB@Fe for 1 h as a control. The excitation wavelength of the HepG2 cells was 405 nm, and the emission was collected from 420 nm to 500 nm.

2.3 Hypoxic conditions in cell and zebrafish incubation

First, 1 mM AA was added to cells and zebrafish. Then, 0.1% O_{2} concentration was generated with AnaeroPack™ (Mitsubishi Gas Chemical Company, Co. Inc., Japan). Also, 1−20% O_{2} concentration was generated with a multi gas incubator (Sanyo) by means of N_{2} substitution.

2.4 Establishment of the liver ischemia mice model and imaging of liver ischemia tissue sections

Six to eight week-old BALB/c mice were obtained from Binzhou Medical University. Mice were group-housed on a 12 : 12 light–dark cycle at 22 °C with free access to food and water (1 mM AA was dissolved in water). BALB/c mice, 20–25 g, were selected and divided into different groups. Mice without any treatment

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were used as the control group. Mice were fasted for 12 h with free access to water before liver ischemic surgery. First, the mice were anesthetized by intraperitoneal injection of 3% pell-tobartitum natricum (80 mg kg⁻¹) dissolved in saline and then, the hairs on the abdomen of the mice were shaved and disinfected using 75% ethanol. The left and middle lobes of the liver were separated after dissecting the abdominal cavity to close the portal vein and hepatic artery using a non-invasive vascular clip. After 0.5 min, the whitened left and middle lobes of the liver were observed to confirm that liver ischemia was successful. Persistent ischemia was observed for 0.5 min, 5 min, 10 min, 30 min and 60 min; then, the ischemic liver tissues were dissected to frozen sections. The entire process of ischemia in mice was conducted on a 37 °C heating plate. Next, the liver ischemia tissue sections were treated with 0.1 mg mL⁻¹ CDs-DB@Fe for 1 h and then washed with PBS buffer (pH = 7.4) to remove excess CDs-DB@Fe. Then, the sections were imaged under a laser scanning confocal microscope. All experimental procedures were conducted in conformity with the institutional guidelines for the care and use of laboratory animals, and the protocols were approved by the Institutional Animal Care and Use Committee in Binzhou Medical University, Yantai, China (Approval Number: No. BZ2014-102R).

3. Results and discussion
3.1 Design strategies and characterization of nanoprobe CD-DB

The synthetic approaches of the multi-response fluorescent nanoprobe CD-DB are outlined in Scheme S1.† The multi-response mechanism for the nanoprobe is shown in Scheme 1. We used a simple one-step method to directly synthesize fluorescent carbon dots using citric acid as the raw material. This is a green chemistry synthesis method because no toxic chemicals were generated. The CDs have a reactive group (–COOH) to provide sites for modification; moreover, the fluorescent CDs have a high quantum yield. Furthermore, desferrioxamine B is a complexing agent that can specifically bind Fe³⁺. We synthesized CD-DB through the reaction between the carboxyl group on CDs and the amino group on desferrioxamine B to form an amide bond. Because of the inner filter effect (IFE), the fluorescence signal of CD-DB could be quenched by Fe³⁺, and Fe²⁺ could be reduced to Fe⁰ via highly reductive AA, which led to the restoration of the fluorescence signal. Therefore, our dual-response nanoprobe CD-DB could detect Fe³⁺ and AA. As is well-known, Fe³⁺ can be reduced by AA, which widely exists in living cells and in vivo. Hence, CDs-DB@Fe obtained from the reaction between CD-DB and Fe³⁺ could be a powerful tool for the detection of AA in living cells and in vivo. The construction of a dual-response nanoprobe has a great potential for the non-invasive detection of AA in vivo and can even be applied in disease detection.

The transmission electron microscopy (TEM) images and mean particle size distribution clearly indicated that the synthesized CD-DB was spherical in shape with an average size of 2.9 nm (Fig. 1a and b). The surface status information on CD-DB was analyzed from the FT-IR spectra (Fig. 1c). The following information was analyzed: the stretching vibrations of C–N at 1422 cm⁻¹ and the vibrational absorption band of C=O at 1679 cm⁻¹. The broad absorption bands at 3100–3500 cm⁻¹ were attributed to the stretching vibrations of O–H and N–H. These absorption bands suggested that many amino functional groups and hydroxyl groups were distributed on the surface of CD-DB, leading to CD-DB having good water solubility.

3.2 Spectroscopic properties toward Fe³⁺ and AA

The previous experiment illustrated that the nanoprobe CD-DB and CDs-DB@Fe would work well under physiological conditions (pH = 7.4) [ESI†]. The pH values hardly affected the fluorescence intensity of CD-DB and CDs-DB@Fe. The results indicated that our nanoprobe would be suitable for applications in living cells and in vivo. As shown in Fig. 2a and b, CD-DB (0.1 mg mL⁻¹) displays obvious absorption and fluorescence emission profiles at 350 nm (ε₃50 nm = 2.9 × 10⁴ M⁻¹ cm⁻¹) and 470 nm, respectively. On increasing the concentrations of Fe³⁺ (0–45 μM) in the CD-DB aqueous solution, the fluorescence signal of CD-DB was quenched due to IFE and formed CDs-DB@Fe. We then established a quantitative linear relationship between the fluorescence intensity (F₄70 nm) and Fe³⁺ concentrations. From Fig. 2c, we can infer that our nanoprobe is suitable for Fe³⁺ detection. The regression equation was F₄70 nm = −2.22 × 10⁴ [Fe³⁺] μM + 1.16 × 10⁶, with r = 0.9930. The limit of detection was 45 nM (3σ/k, where σ is the standard deviation of the blank measurement, and k is the

![Scheme 1](image)

**Scheme 1** The multi-response mechanism for the nanoprobe.

![Fig. 1](image)

**Fig. 1** Characterization of nanoprobe CD-DB. TEM imaging (a) and the mean particle size distribution (b) of CD-DB. (c) FT-IR spectrum of nanoprobe CD-DB.
Fe$^{3+}$ and AA on the fluorescence intensity of the nanoprobe are 4.57 × 10$^4$, with AA concentrations (Fig. 2e), which revealed the ability of CDs-DB@Fe for the detection of AA, the fluorescence emission limit was 0.1 μM. It is noteworthy that the quenched fluorescence of CDs-DB@Fe could be recovered upon the addition of AA. Thus, CDs-DB@Fe was incubated with increasing concentrations of AA (10–100 μM). As shown in Fig. 2d, since Fe$^{3+}$ is reduced to Fe$^{2+}$ and the IFE process is removed, the fluorescence maximum at 470 nm strongly increases. To assess the ability of CDs-DB@Fe for the detection of AA, the fluorescence emission at 470 nm was observed to exhibit a linear relationship with AA concentrations (Fig. 2e), which revealed the ability of CDs-DB@Fe for the quantitative and qualitative detection of AA. The regression equation was $F_{470\text{nm}} = 1.05 × 10^4 [\text{AA}] \mu\text{M} + 4.57 × 10^4$, with $r = 0.9959$. The limit of detection was calculated to be 80 nM (3σ/k), and the experimental detection limit was measured to be 0.2 μM under the experimental conditions. The photographs of the effect of different concentrations of Fe$^{3+}$ and AA on the fluorescence intensity of the nanoprobe are shown in Fig. 2b and d. These results demonstrated that our nanoprobe could monitor Fe$^{3+}$/AA concentrations, which made our nanoprobe a potential tool for applications in cells.

3.3 Selectivity to Fe$^{3+}$ and AA

To verify the fluorescence response to other metal ions, we tested the selectivity of CD-DB against common metal ions and that of CDs-DB@Fe against amino acids and vitamins in HEPES solutions (10 mM, pH 7.4). Compared to other metal ions, CD-DB offered remarkable fluorescence quenching for Fe$^{3+}$. Fig. S3† demonstrates that Cd$^{2+}$, Zn$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, Pb$^{2+}$, Fe$^{3+}$, Cu$^{2+}$, Ag$^+$, Mg$^{2+}$, Mn$^{2+}$ and Hg$^{2+}$ cause almost no response in the emission signal after 20 min incubation. The results showed that CD-DB had good selectivity for Fe$^{3+}$ detection over other common metal ions. The fluorescence responses of CDs-DB@Fe to other amino acids and vitamins were also evaluated (Fig. 2f). CDs-DB@Fe (0.1 mg mL$^{-1}$) could provide an obvious fluorescence response to AA. However, no obvious changes in the spectra were observed upon the addition of vitamin A, vitamin B1, vitamin B2, vitamin B6, biotin, folic acid, vitamin B12, inositol, vitamin D, tocopherol, vitamin K, glutathione, l-cysteine, tryptophan, glycine, alanine, cystine, leucine, l-serine and histidine. As shown in Fig. S4,† no obvious changes in the fluorescence intensity can be observed upon the addition of OH$^-$, NO$^-$, SO$_4^{2-}$, HSO$_4^-$, CO$_3^{2-}$, HCO$_3^-$, SO$_3^{2-}$, HSO$_3^-$, ClO$^-$, PO$_4^{3-}$, F$^-$, C$^-$, Br$^-$ and I$^-$. These results demonstrated that CDs-DB@Fe was a highly selective fluorescent nanoprobe for AA detection over other amino acids, anions and vitamins. Taken together, these kinetic and selectivity assays revealed that our nanoprobe could work well under physiological conditions for the investigation of Fe$^{3+}$ and AA.

3.4 Imaging of cell response to Fe$^{3+}$ and AA and organelle localization

Encouraged by the sensitive and selective detection of Fe$^{3+}$ and AA with our nanoprobe, we tried to exploit the potential applications for Fe$^{3+}$ and AA detection in cells and in vivo. Therefore, we first performed a standard MTT assay to evaluate the cytotoxicity of CD-DB and CDs-DB@Fe. As demonstrated in Fig. S5 and S6,† more than 90% of the HepG2 cells survived after the cells were incubated with CD-DB (0–200 μg mL$^{-1}$) for 24 h; moreover, more than 80% of the HepG2 cells survived after the cells were incubated with CDs-DB@Fe (0–200 μg...
mL⁻¹). The 50% cell survival concentration value (IC₅₀) was predicted to be 2.0 mg mL⁻¹ for CD-DB and 1.7 mg mL⁻¹ for CDs-DB@Fe, which indicated the low cytotoxicity of CD-DB and CDs-DB@Fe.

We next investigated the ability of CD-DB for Fe³⁺ and AA detection in living cells. The HepG2 cell line was chosen as the test model. First, the cells were incubated with 10 μg mL⁻¹ CD-DB for 1 h at 37 °C as a control. The cells showed a strong fluorescence signal (Fig. 3a) and then, 50 μM Fe(NO₃)₃ was added to the cells and treated for 10 min. As shown in Fig. 3a, the cells display apparently quenched fluorescence, which indicated that CD-DB could detect the additional Fe³⁺ ions in living cells. Next, the cells were incubated with 50 μM AA for another 10 min, and the fluorescence intensity rose to a plateau rapidly (Fig. 3a). Then, the cells were treated with CD-DB under continuous laser irradiation for 1 h, and the cells still exhibited strong fluorescence intensity. These results indicated that CD-DB could be successfully applied to monitor Fe³⁺ and AA, and CD-DB also had good light stability in living cells.

To verify how CD-DB was internalized by the cells, a co-localization experiment was carried out by co-staining the HepG2 cells with CD-DB and LysoTracker Green DND-26 (a commercial Lyso-Tracker). The tested cells were incubated with 10 μg mL⁻¹ CD-DB for 1 h at 37 °C before imaging; then, the cells were treated with 1 μg mL⁻¹ LysoTracker for 15 min. For the CD-DB fluorescent channel, a strong fluorescence signal appeared in the cells. Lysosomes were stained by LysoTracker and exhibited a clear fluorescence signal at the green channel (570–650 nm) under the excitation of 559 nm. The merged image of the CD-DB fluorescent channel and lysosomes mainly shows an orange fluorescence signal (Fig. 3b), which indicates that the fluorescence signals at the two channels overlap well in discrete subcellular locations. We acquired the Pearson’s coefficient (r = 0.95), implying the preferential distribution of CD-DB in lysosomes. Simultaneously, the intensity profiles of the linear regions of interest (white line in Fig. 3b) across the HepG2 cells were in close synchrony. A large amount of CD-DB was observed to accumulate in the lysosomes in the cells, indicating that CD-DB might be ingested via endocytosis.

Since AA cannot be generated in living cells and in vivo, we added 1 mM AA to the cells before imaging and employed the nanoprobe CDs-DB@Fe to study its physiological function. Pan et al. reported the metabolic way of AA under normoxia and hypoxia conditions. As is well-known, AA is a reducing agent (antioxidant) that can neutralize free radicals and relieve oxidative stress. Therefore, CDs-DB@Fe was employed as the nanoprobe to detect the fluctuation of AA under a hypoxia condition. Initially, the HepG2 cells were cultured under hypoxic conditions with 20%, 10%, 5%, 1%, and 0.1% O₂ for 4 h; then, the cells were incubated with 10 μg mL⁻¹ CDs-DB@Fe for 1 h at 37 °C. As shown in Fig. 3c, strong fluorescence images were acquired from the cells cultured with 20% and 10% O₂, while the signal was quite weak under 1% and 0.1% O₂ conditions.

These results revealed that the content of AA decreased in living cells under hypoxic conditions due to the consumption of free radicals. A laser scanning confocal microscope is valuable for relatively small amounts of cells only in visual fields, which might decrease the reliability of the data owing to various uncontrolled factors. A flow cytometry assay can provide more statistically reliable data by analyzing millions of cells rapidly and sensitively, which has been highly needed. Furthermore, all the fluorescence data were verified via a flow cytometry assay (Fig. 3c). In addition, the expression of the hypoxia inducible factor 1α (HIF-1α) was examined using western blot analysis to exhibit the hypoxic cell model. The expression of HIF-1α was evidently upregulated under hypoxia stress (Fig. 3d). These data demonstrated that CDs-DB@Fe was
valuable for the detection of intracellular AA, and AA decreased under hypoxic conditions.

3.5 Imaging of zebrafish under hypoxic conditions

Since CDs-DB@Fe was successfully applied to monitor AA in living cells, we next strived to investigate the detection of AA in larval (7 days post fertilization) zebrafish under hypoxic conditions. At this stage in their development, zebrafish were approximately 3 mm in length and maintained a high level of transparency. We first tested the toxicity of CDs-DB@Fe for zebrafish. By monitoring the zebrafish for 7 days (Fig. 4b), it was observed that no zebrafish died after they were incubated with 1 mg mL\(^{-1}\) CDs-DB@Fe, which indicated the negligible toxicity of CDs-DB@Fe. The zebrafish models were incubated with a multi-gas incubator (Sanyo) by means of N\(_2\) substitution under 20\%, 10\%, 5\% and 1\% O\(_2\) for 4 h. Then, the zebrafish were incubated with 0.1 mg mL\(^{-1}\) CDs-DB@Fe for 1 h. The imaging of zebrafish was obtained by a laser scanning confocal microscope. As expected, the zebrafish under 20\% and 10\% O\(_2\) showed a strong fluorescence signal, and the fluorescence signal significantly decreased under 5\% and 1\% O\(_2\). All these results demonstrated that AA decreased in the zebrafish under hypoxic conditions due to the hypoxia-induced oxidative stress. In addition, our nanoprobe CDs-DB@Fe was found to be suitable for imaging AA in zebrafish.

3.6 Toxicity of CDs-DB@Fe in mice

In view of the excellent biocompatibility of CDs-DB@Fe in zebrafish, BALB/c mice were selected as the models for evaluating the toxicity of CDs-DB@Fe in vivo. First, a 200 µL PBS solution (pH = 7.4) was injected into the mice once a day by intravenous injections and this was continued for 30 days (control group). In addition, 200 µL CDs-DB@Fe (0.4 mg kg\(^{-1}\)) was injected into a separate group of mice once a day by intravenous injections and this was continued for 30 days (experimental group). Then, the mice were sacrificed, and the main tissues and organs were removed for H&E staining. The CDs-DB@Fe-treated group and PBS group exhibited no distinct damage against the normal tissues (heart, liver, spleen, lung and kidney) (Fig. 5b). Hence, it was clear that CDs-DB@Fe had low toxicity and unobvious side effects in vivo.

3.7 Fluorescence imaging of liver ischemia tissue

In an actual biological sample, ischemia is the main reason for hypoxia. Therefore, AA in the ischemia organs should be evaluated to expand the application of our nanoprobe. The liver ischemia mice were selected as the test model. The liver was persistently ischemic for 5 min, 10 min, 30 min and 60 min by closing the portal vein with a non-invasive vascular clip. The mice without any treatment were used as the control group. All mice were sacrificed, and the normal liver tissue...
and ischemic liver tissue treated for different times were dissected for frozen sections. Next, the liver ischemia tissue sections were incubated with 0.1 mg mL$^{-1}$ CDs-DB@Fe for 1 h at 4 °C. Then, the stained sections were imaged by a laser scanning confocal microscope. As shown in Fig. 6b, the control group displays a strong fluorescence signal, and the ischemic liver tissue displays weak fluorescence. As the ischemia time increased, the fluorescence signal became weaker, indicating that CDs-DB@Fe could respond to AA in the liver ischemia conditions. As shown in Fig. 6d, these results suggested that AA decreased in the ischemia-induced hypoxic tissues and persistent ischemia could damage healthy tissues.

4. Conclusions

In summary, we successfully developed a fluorescent nanoprobe CD-DB by connecting CDs and desferrioxamine B for the associated detection of Fe$^{3+}$ and AA. CD-DB exhibited outstanding sensitivity and selectivity towards Fe$^{3+}$ and AA. The bioassays fully demonstrated that CD-DB could associatively detect additional Fe$^{3+}$ and AA in living cells. Furthermore, CDs-DB@Fe could successfully determine the fluctuation of AA in hypoxic cell models and hypoxic zebrafish models. Moreover, a liver ischemia-induced hypoxic mice model was established to monitor the changes in AA. These results indicated that AA was consumed in various hypoxic models as AA could neutralize the hypoxia-induced free radicals and relieve oxidative stress. As far as we know, this is the first time that a CD-based nanoprobe has been applied to analyze the changes in AA in a hypoxic biological sample. In addition, our nanoprobe also exhibited low toxicity for living cells, zebrafish and mice. Overall, our nanoprobe as an ideal nanoagent has a great potential to understand the physiological effect of AA.

Conflicts of interest

There are no conflicts to declare.

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