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2020

Feng, L., Gai, S., He, F., Yang, P. & Zhao, Y. (2020). Multifunctional bismuth ferrite nanocatalysts with optical and magnetic functions for ultrasound-enhanced tumor theranostics. *ACS Nano*, 14(6), 7245-7258. <https://dx.doi.org/10.1021/acsnano.0c02458>

<https://hdl.handle.net/10356/146293>

<https://doi.org/10.1021/acsnano.0c02458>

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Multifunctional Bismuth Ferrite Nanocatalysts with Optical and Magnetic Functions for Ultrasound Enhanced Tumor Theranostics

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ABSTRACT: Ultrasound (US) assisted oncotherapy has aroused extensive attention due to its capacities to conquer significant restrictions covering short tissue penetration depth and high phototoxicity in photo-induced therapy. We herein developed a class of pure phase perovskite-type bimetallic oxide, namely bismuth ferrite nanocatalysts (BFO NCs), for multimodality imaging-guided and US-enhanced chemodynamic therapy (CDT) against malignant tumor. As-prepared BFO nanoparticles with poly(ethylene glycol)-grafted phosphorylated serine (pS-PEG) modification exhibit satisfactory physiological stability and biocompatibility. The BFO NCs also present high fluorescence emission within the second near-infrared region when irradiated upon 808 nm laser. Intriguingly, the BFO NCs demonstrate highly efficient US-enhanced generation of hydroxyl free radicals, as the cavitation bubbles produced by US trigger partial grievous turbulence and promote the transfer rate of the Fenton reagents. Thus, the BFO NCs enable effective inhibition of tumor growth assisted with external US, and the treatment efficacy can be monitored by computer tomography, magnetic resonance and fluorescence imaging. Meanwhile, H₂O₂ and US, as double logic gate, activate the BFO NCs to trigger the iron-catalyzed and US-enhanced CDT with high specificity and treatment efficiency. Therefore, the BFO NCs as a theranostic agent with an enhanced chemodynamic therapeutic effect assisted with external US and a multimodality imaging capacity are put forward, which show a promising prospect for noninvasive chemodynamic oncotherapy.

KEYWORDS: bismuth ferrite, chemodynamic therapy, multimodality imaging, nanocatalysts, ultrasound

Development of treatment modalities with simultaneously high treatment efficacy and negligible side effects is desired in conflicting malignant tumor.¹⁻⁵ Chemodynamic therapy (CDT) as an emerging therapeutic approach, utilizing the reaction between Fe-containing nanocatalysts and overexpressed hydrogen peroxide (H_2O_2) in tumor sites to generate hydroxyl free radical ($\bullet OH$), was defined as *In Situ* treatment.⁶⁻⁹ The nanostructures that can be responsive to peculiar tumor microenvironment (TME) not only possess high functionality and selectivity, but also insignificant invasiveness.¹⁰⁻²² Unluckily, the therapeutic efficacy of CDT is confined due to the inadequate Fenton reaction efficiency in weakly acidic TME, on account of the rigorous Fenton reaction condition, limiting its further biological applications.²³⁻²⁶ As a consequence, how to increase the therapeutic efficacy of Fenton reaction in tumor is the primary problem of CDT at present.

Ultrasound (US) is a mechanical sound wave with periodic continuous vibrations, which has been extensively applied in biomedical science, such as a routine use for diagnostic US imaging of soft tissue, controlled drug release during chemotherapy, ablating tumor by US absorption hyperthermia, and causing sonodynamic therapy.²⁷⁻³² It is important that the US irradiation is conducive to the generation of reactive oxygen species (ROS) for triggering the tumor cell death, which may be an efficient way to enhance the Fenton reaction efficiency.³³⁻³⁵ A major strength of US-based cancer therapy is its excellent tissue-penetrating ability when using US as the irradiation source in comparison with light-induced therapy.³⁶⁻³⁸ Consequently, if the enhancement effect of US irradiation on Fenton performance is combined with special Fenton reagent, the treatment effect of CDT may be satisfactory.

As is known to all, perovskite-type mixed oxides (ABO_3) as heterogeneous catalysts are significant for industrial reactions.³⁹⁻⁴¹ Bismuth ferrite (BFO) magnetic nanoparticles as a type of the ABO_3 exhibit an application potential for memory and logic devices.⁴²⁻⁴⁶ BFO nanoparticles can also perform as a nanocatalyst for photocatalysis.⁴⁷⁻⁵¹ Because BFO concurrently contains the Bi and Fe elements as well as perovskite-type structure, BFO nanoparticles show a potential prospect for catalytic excitation of H_2O_2 and multimodality imaging,⁵²⁻⁵⁶ thus offering an exciting opportunity for imaging-guided tumor diagnosis and treatment through the specific performance of BFO magnetic nanoparticles. Until now, reports on developing BFO-based nanosystems for cancer treatment, not to mention on US-assisted CDT, are still rare.

Scheme 1. Schematic illustration of BFO NCs for US-enhanced CDT of cancer.

For the purpose in view, we developed the pure phase BFO magnetic nanoparticles modified by poly(ethylene glycol)-grafted phosphorylated serine (pS-PEG) as “all-in-one” multifunctional theranostic nanocatalysts (BFO NCs) for imaging-guided highly efficient antitumor therapy, which display outstanding Fenton effect (**Scheme 1**). The treatment effect of the Fenton reaction could be apparently enhanced by external US assistance. The BFO NCs comprehended high fluorescence emission intensity within second near-infrared (NIR-II) regions when irradiated upon 808 nm laser. Furthermore, the prominent NIR-II fluorescence emission, intrinsic magnetic performance, and effective X-ray attenuation coefficient ($5.74 \text{ cm}^2/\text{kg}$ at 100 kV) of Bi endow BFO NCs with a high capacity as contrast agents for fluorescence imaging, computed tomography (CT) imaging, and magnetic resonance imaging (MRI). Effective inhibition of the tumor growth was displayed according to the *In Vitro* and *In Vivo* experiment results, which attributed to the US-enhanced CDT. Therefore, H₂O₂ and US, as a “double” logic gate, activate BFO NCs to induce the iron-catalyzed US-enhanced CDT with significant specificity and treatment efficiency.

Figure 1. Synthesis and basic characterizations of BFO NCs. (a) Schematic illustration for the preparation process of BFO NCs. (b) Crystal structure of BFO (purple, yellow, and red represent Bi, Fe, and O atom, respectively). (c) TEM image of as-synthesized BFO. (d) High-resolution TEM image of BFO. Inset: electron-diffraction patterns confirming that BFO has the rhombohedral R3c nanostructure. (e) PXRD patterns of BFO and the standard card (JCPDS: 20-0169). (f) Energy dispersed spectrum and (g) high-angle annular dark-field scanning TEM image along with elemental mapping of Bi, O, and Fe of BFO. Scale bar: $50\ \text{nm}$.

RESULTS AND DISCUSSION

Figure 1a exhibits the synthetic procedure of BFO NCs for US enhanced Fenton effect. BFO was prepared by a sol-gel approach, and modified with pS-PEG to enhance physiological stability and biocompatibility (abbreviated as BFO NCs, **Figure 1b**). The as-synthesized BFO NCs are nanosized particles with an average size of $48.7\ \text{nm}$, investigated by typical transmission electron microscopy (TEM, **Figure 1c**). The high-resolution TEM (HR-TEM) image of the BFO is also

clearly presented along with the electron diffraction pattern, as demonstrated in **Figure 1d**. The regular spacing of the researched lattice from BFO is 0.386 and 0.279 nm, in accordance with the (012) and (104) crystal planes, respectively. These results verify that the BFO NCs possess a single-phase perovskite nanostructure (**Figure 1b**). For comparison, the TEM images of BFO calcined at various temperatures were also presented (**Figure S1**). Powder X-ray diffraction (PXRD) patterns of the BFO with various calcination temperatures are shown in **Figure 1e** and **Figure S2**. The nanocatalysts are formed when the sintering temperature is above 500 °C, in which the characteristic diffraction peaks at 22.48°, 31.89°, and 39.18° should be inevitably attributed to the (102), (104), and (006) planes of BFO NCs (JCPDS card No. 20-0169), respectively. No additional diffraction peaks assigned to Bi₂O₃/Fe₂O₃ and Bi₂Fe₄O₉ was inspected, confirming that the obtained BFO has a pure-phase perovskite nanostructure. Two distinct characteristic absorption peaks were detected at about 553 and 443 cm⁻¹ in Fourier transform infrared (FTIR) spectra of BFO calcined at different calcining temperatures (more than 500 °C), suggesting Fe–O stretching and O–Fe–O bending vibration of the FeO₆ species in BFO nanoparticles, separately (**Figure S3a**).

Furthermore, the Raman scattering spectra of BFO calcined at varied temperatures were given in **Figure S4**. The Raman spectral results indicate the generation of the rhombohedral perovskite structure (R3c) for BFO, which are consistent with other reported results.⁵⁷ Additionally, the chemical valence of the obtained BFO was evaluated using X-ray photoelectron spectroscopy (XPS, **Figure S5a** and **S6**). Two signal peaks of Bi 4f_{7/2} and Bi 4f_{5/2} at 158.71 and 164.01 eV could be distinguished in the high-resolution XPS spectrum of the Bi 4f state, respectively. The energy difference between these two characteristic peaks of Bi 4f is 5.3 eV, affirming the existence of both Bi³⁺ ion and Bi–O bond, as presented in **Figure S5b**. The XPS high-resolution spectrum of Fe 2p state was also presented in the range of 740-700 eV. The Fe 2p_{3/2} and Fe 2p_{1/2} species situated at the binding energy of 710.61 and 724.16 eV could be ascribed to the Fe–O bond, respectively. A satellite peak observed at the position higher than 8.35 eV of the Fe 2p_{3/2} principal peak should be assigned to the Fe³⁺ species. On account of the different electron configuration of *d* orbital, Fe³⁺ and Fe²⁺ species would appear at 6 eV or 8 eV above the 2p_{3/2} signal peak (**Figure S5c**).⁵⁸ The above analysis demonstrates that the oxidation state of the Fe element in BFO NCs is comprised of Fe²⁺ and Fe³⁺ species with an approximate ratio of 1:1.6. In the O 1s high-resolution spectrum, there are mainly three characteristic peaks at about 529.53,

531.61 and 532.70 eV, which are contributed to evident O species, *i.e.*, O defects, O²⁻ ion in Bi–O and Fe–O bonds of the nanostructure, and O in groups such as –CO₃ and –OH, respectively (**Figure S5d**). Hence, the Bi and Fe valence acquired by the XPS study is accordance with BFO in previously reported literature.⁵⁹ Moreover, X-ray energy dispersive spectroscopy (EDS) spectrum affiliated to TEM was utilized to assess atomic ratio of Fe and Bi in the synthetic materials. The analysis was quantitatively performed by taking the mean values of five different samples, showing that the atomic ratio of Fe to Bi is approximately 1:1 within the instrumental accuracy, as displayed in **Figure 1f**. Such result was further verified by inductively coupled plasma mass spectrometry (ICP-MS), presenting that the ratio of Fe to Bi was 1.00:0.99 (Table S1). The elemental mapping of BFO by EDS reveals that uniform distribution of Fe, Bi, and O elements throughout the whole nanostructure, confirming the successful fabrication of the BFO nanostructure (**Figure 1g**).

The magnetization *versus* magnetic field (Oe) curve of BFO magnetic nanocatalysts was measured. As shown in **Figure S7**, the BFO with calcination temperature of 500 °C revealed that the maximum saturation moment per unit mass is approximately 0.68 emu g⁻¹. Superparamagnetic performance of the nanocatalysts imparts easy recovery of magnetic property after the usage. The presence of ferromagnetism is attributed to spatial transformation of the spin structure.⁶⁰ Moreover, UV–vis absorption spectra of these materials were investigated and compared. No significant absorption in NIR region could be detected (**Figure S8a**). Then, the optical performance of BFO with varied calcination temperatures was investigated by UV-vis diffuse reflectance spectroscopy (**Figure S8b**). The BFO NCs exhibit an apparent absorbance in the range of UV–vis–NIR region. The band gap value of BFO calcined at 500 °C was determined on the basis of Kubelka–Munk function method, which is calculated to be 1.67 eV and lower than the reported literature (**Figure S8c,d and S9**), confirming that it is a promising nanomaterial for bio-application. Ultimately, the BFO nanoparticles calcined at 500 °C for 2 h were selected for further characterization and experiments. After modified with pS-PEG, the hydrodynamic diameter of the BFO NCs is about 78.8 nm, which is higher than the diameter (**Figure S10**) determined by TEM owing to the presence of polymer chains. The polydispersity index value from the dynamic light scattering result is 0.147. The achieved BFO NCs demonstrated tremendous dimensional stability when were dispersed in various solvents, including deionized water, cell culture medium, phosphate buffered saline (PBS), and serum (**Figure S11a,b**). There

was no apparent change in the UV–vis absorption spectra and size distribution of BFO NCs in different conditions even during the 14-day culture process (**Figure S11c,d**). The changes in zeta potential (**Figure S12**) among bare BFO nanoparticles (1.6 ± 0.5 mV) and BFO NCs (-9.3 ± 0.8 mV) validate the successful modification of pS-PEG, which also confirmed by FTIR (**Figure S3b**). Then, we adopted thermogravimetric analysis (TGA) to measure the pS-PEG content in the BFO NCs, and the calcined BFO nanoparticles were utilized as control. **Figure S13** exhibits the TGA result of the BFO NCs in the temperature range of 30–800 °C, acquiring a pS-PEG loading capacity of wt.5.6%. Based on the above analysis, the successful surface modification of pS-PEG on BFO NCs was well certified.

Figure 2. (a) Excitation spectrum of BFO NCs ($\lambda_{em} = 394$ nm). Inset: photographs of the BFO NCs without and with ultraviolet light irradiation (showing luminescence). Fluorescence emission spectra of BFO NCs when irradiated with (b) xenon lamp ($\lambda_{ex} = 284$ nm) and (c) laser of

808 nm with 0.92 W/cm² power density. Fluorescence lifetime decay curves for the emission of BFO NCs at the wavelength of (d) 284, (e) 394, and (f) 1250 nm. The powder samples of BFO NCs were used for fluorescence detection.

According to the narrow bandgap energy (about 1.67 eV) and obvious absorption peaks of BFO within the range of 200–900 nm (**Figure S8** and **S9**), BFO NCs could effectively absorb NIR light. However, no significant hyperthermia effect could be detected when irradiation upon NIR laser (**Figure S14**). Thus, the absorbed energy may be converted into other forms. Hence, we further explored the fluorescence properties of BFO NCs. The excitation spectrum of BFO NCs was examined, and an obvious characteristic peak at 248 nm was found under the emission wavelength of 394 nm. The insert in **Figure 2a** shows a luminous photograph of the BFO NCs under ultraviolet light irradiation. Then, the fluorescence spectra of BFO NCs at varied calcined temperatures were measured (**Figure 2b,c**). Special fluorescence emission spectra of BFO NCs in visible region were detected with Xenon lamp excitation (wavelength: 248 nm). The blue fluorescence emission at the central wavelength of 284 and 394 nm can be attributed to the $^3P_1 \rightarrow ^1S_0$ transition of Bi^{3+} , which is basically consistent with those reported results.⁶¹ For BFO NCs with varied calcination temperatures, there is no significant change in fluorescence emission intensity. Photographs in the inset of **Figure 2a** intuitively demonstrate the fluorescence changes after irradiation with UV lamp. Fluorescence emission in the second near-infrared window of 1000–1700 nm possesses the features of deeper tissue penetration depth, more efficient signal-to-noise ratio, and higher spatial resolution, exhibiting a potential capacity for fluorescent imaging. Therefore, the fluorescence spectra of BFO NCs excited by laser of 808 nm were conducted. Apparently, a broadband near infrared fluorescence emission peak in the 950–1600 nm range can be found (**Figure 2c**), and the fluorescence emission center is located at 1250 nm. The emission peak of bismuth-doped nanomaterials in the near infrared luminescence region may be attributed to the low-valence bismuth, bismuth atomic cluster or defects between bismuth and other substances. Because the energy level structure of bismuth is very complex, the near infrared luminescence mechanism of bismuth is not clear up to now and needs further exploration. A schematic diagram of possible bismuth ion energy levels is presented as a reference (**Figure S15**). Furthermore, the fluorescence lifetime decay curves for the emission peaks of BFO NCs at the wavelength of 284, 394, and 1250 nm were carried out, as shown in **Figure 2d-f**. It is extremely

clear that the corresponding fluorescence lifetimes of BFO NCs at different emission positions are 1.5, 112.3, and 29.4 μ s, respectively. Based on the special fluorescence property of BFO NCs, an application prospect in biological imaging was expected.

Figure 3. Studies on catalytic performance of BFO NCs. Time-course absorbance at 653 nm *versus* (a) varied H_2O_2 concentrations (100, 50, 25, and 12.5 mM) and (d) US time (15, 30, 60 and 120 s) under 0.8 mM TMB at pH = 6.0. (b,e) Michaelis-Menten and (c,f) Lineweaver-Burk fitting curves of initial hydroxyl radical generation velocity *versus* H_2O_2 concentration and US time having 0.8 mM TMB at pH = 6.0. (g) Lineweaver-Burke fitting curves under 0.8 mM TMB at varied H_2O_2 concentrations and US time. (h) ESR spectra recorded at stated measurement conditions. (i) Schematic comparison of the classical Fenton reaction and the US-enhanced Fenton reaction. Error bars and mean values are defined as S.D. and mean, respectively.

The production of $\bullet\text{OH}$ free radical in the Fenton reaction process is a significant element for CDT. Therefore, the $\bullet\text{OH}$ generation capacity of BFO NCs was detected by utilizing the chemical probe methylene blue (MB) in phosphate buffer with varied pH of 6.0 and 7.4, where the absorption intensity decrease at 653 nm is induced by $\bullet\text{OH}$ radical. The concentration-dependent generation of $\bullet\text{OH}$ is shown in **Figure S16a-d**, which progressively increases with the elevation level of H_2O_2 in acidic condition, confirming the occurrence of Fenton reaction. After stimulation upon external US, the absorption strength of the solution is apparently lower than that without US illumination, demonstrating that the sound waves achieved from BFO NCs with US irradiation can efficaciously enhance the production of $\bullet\text{OH}$. Also, a significant decrease in absorbance intensity of MB is shown, indicating that the $\bullet\text{OH}$ free radicals could be effectively generated with increasing incubation time, as displayed in **Figure S16e-g**. We further investigated the influence of US time and power density on the generation of $\bullet\text{OH}$ free radical (**Figure S16h,i**). With prolonging the time of US irradiation, the absorption intensity of MB significantly decreases. Since the transfer rate of nanosystems as Fenton reaction reagents could be accelerated by US treatment, the yield capacity of $\bullet\text{OH}$ radical is obviously enhanced upon increasing the US power density, prominently improving the efficiency of Fenton reaction. More interestingly, the reclaimed BFO NCs have a high chemo-catalytic activity and can be used repeatedly (**Figure S17**).

To further investigate the $\bullet\text{OH}$ generation capacity of BFO NCs during the US-enhanced CDT process, 3,3',5,5'-tetramethyl-benzidine (TMB) was adopted to measure free radical generation. The colorless TMB could be oxidized to color TMB cation by the yielded $\bullet\text{OH}$ in the presence of H_2O_2 and BFO NCs under acidic condition, exhibiting apparent absorbance at 653 nm. On the basis of the above-mentioned theories, representative Michaelis–Menten kinetics was used to research catalytic properties of BFO NCs. H_2O_2 (12.5, 25, 50, and 100 mM), US time (15, 30, 60, and 120 s), and 200 $\mu\text{g/mL}$ BFO NCs were used as the experiment conditions during the studies. The time-dependent absorbance by adding of H_2O_2 with different concentrations into the buffer solution of BFO NCs with the pH value of 6.0 was measured (**Figure 3a-c**), and homologous average initial reaction velocity was estimated. The average initial velocity of absorbance change is transformed to the initial velocity (v_0) of $\bullet\text{OH}$ formation by the Beer–Lambert law, which is plotted *versus* paralleling concentrations ($[S]$) and fitted with Michaelis–Menten curves (equation 1, **Figure 3b**). Photographs inserted in **Figure 3b** intuitively exhibit the chromogenic variations

after 1000 s upon H₂O₂ accretion with different concentrations. Furthermore, to standardize the maximum velocity ($V_{\max}$) and Michaelis–Menten constant (K_M), a linear Lineweaver–Burk plot (equation 2) was achieved (**Figure 3c**). The $V_{\max}$ and K_m data were computed to be 12.45 mM and $1.244 \times 10^{-8} \text{ M s}^{-1}$ for BFO NCs. Analogously, in a forthcoming experiment for BFO NCs with various US time, the steady-state kinetics of BFO was also studied. The US time-dependent absorbance intensity was recorded in **Figure 3d** and the initial velocities with homologous US time were acquired and plotted according to Michaelis–Menten and Lineweaver–Burk equations (**Figure 3e,f**). The K_m and $V_{\max}$ of BFO NCs were estimated to be 14.90 mM and $2.109 \times 10^{-8} \text{ M s}^{-1}$, respectively.

$$v_0 = \frac{V_{\max} \cdot [S]}{K_M + [S]} \quad (1)$$

$$\frac{1}{v_0} = \frac{K_M}{V_{\max}} \cdot \frac{1}{[S]} + \frac{1}{V_{\max}} \quad (2)$$

Meanwhile, the generation of •OH free radical was quantitatively evaluated *via* electron spin resonance (ESR) measurement (**Figure 3h**). 5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO) was utilized to capture •OH free radical, and the generated free radical spin adducts could be inspected through ESR. In comparison with H₂O₂ or BFO alone, more obvious DMPO-OH characteristic peaks (1:2:2:1) could be easily recognized in acidic rather than neutral condition. Significantly enhanced characteristic signal peaks were observed in the BFO with US irradiation group, indicating that the generation of •OH radical was efficiently enhanced by the US irradiation. The possible mechanism was presented as below (**Figure 3i**). The conversion efficiency of Fe³⁺ to Fe²⁺ could be expedited by means of US irradiation, and the produced Fe²⁺ further interacts with H₂O₂ to afford more •OH free radicals. The use of US in conjunction with powerful shockwave from the cavitation bubbles produced by US could trigger partial violent turbulence, and expedite the mass transfer rate of the nanosystems as Fenton reaction reagents.²⁸ Therefore, we believe that BFO NCs with special performance could be utilized as an efficient Fenton agent to yield •OH and kill tumor cells, further improving the therapeutic effect by US irradiation.

Figure 4. (a) Cytotoxicity of BFO NCs toward HeLa cells at different pH (6.0 and 7.4) and H_2O_2 concentrations (50 and 100 μM). (b) Cell viability of HeLa cells incubated with BFO NCs having different treatments (pH = 6.0 and the concentration of H_2O_2 is 50 μM). (c) Relative viability of HeLa cells under US-enhanced CDT with BFO NCs for varied US irradiation durations ($n = 3$, mean $\pm$ SD). (d) CLSM images of HeLa cells after co-incubation with FITC-labelled BFO NCs for 0.5 h and 3 h. (e) Schematic comparison of the classical US-enhanced Fenton reaction in tumor cells. (f) CLSM images of HeLa cells stained by Calcein AM/PI and DCFH-DA under different treatments (Green fluorescence: Calcein AM for living cells; red fluorescence: PI for dead cells). Scale bar = 50 μm . *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$.

Before affirming catalytic performance of the BFO NCs *In Vitro*, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was provided to investigate the cytotoxicity. Relative cell viabilities of L929 cells were more than 93% when incubated with BFO NCs having the concentration up to 800 $\mu\text{g/mL}$ for 24 h or 48 h (**Figure S18a**). Similarly, BFO NCs displayed no evident cytotoxicity effects to HeLa cells after 24 h of

cultivation at the corresponding concentrations (**Figure S18b**). The cytotoxicity of the varied H_2O_2 concentration and US power density was also investigated in detail. Negligible cell viability change by the addition of H_2O_2 at various concentrations or US with different power densities was observed (**Figure S18c,d**). To further achieve more complete understanding of the cytotoxicity performance of BFO NCs, HeLa cells were treated with BFO NCs at different concentrations of 12.5, 25, 50, 100, 200, 400, and 800 $\mu\text{g/mL}$ under neutral (pH 7.4) and acidic (pH 6.0) experimental conditions in the presence of H_2O_2 (50 or 100 μM) for 24 h (**Figure 4a**). The cell viability was evaluated by a standard MTT method. It can be found that the cell survival rate depends on the pH value, H_2O_2 and sample concentrations simultaneously. In the cytotoxicity assay, the cell viabilities decrease significantly as the sample concentration increases under the acidic culture medium. However, there was no significant decrease in cell survival rate at corresponding concentrations under the neutral condition. In any case, with the same concentrations of H_2O_2 and BFO NCs, the acidic microenvironment triggers apparently higher toxicity than that under the neutral condition. We further researched the effect of US on the cell viability (**Figure 4b,c**). HeLa cells incubated with BFO NCs under US treatment exhibited an apparent decline in cell viability. Meanwhile, with the prolonged US treatment time, the activity of HeLa cells significantly dropped, indicating that the considerable therapeutic efficiency of CDT could be enhanced by US.

Moreover, confocal laser scanning microscopic (CLSM) images were adopted to display that BFO NCs could efficiently accumulate in tumor cells, as observed by intracellular green fluorescence originated from fluorescein isothiocyanate (FITC) modified BFO NCs after co-incubation with cancer cells (**Figure 4d**). As the cultivate time goes by, the signal intensity of green fluorescence evidently enhances (3 h co-incubation), demonstrating that BFO NCs can be effectively ingested by tumor cells. The result was also certified by the bio-TEM images as exhibited in **Figure S19**. The generation of $\bullet\text{OH}$ was verified by using a chemical detector 2',7'-dichlorofluorescein diacetate (DCFH-DA). As compared with the control group, HeLa cells incubated with BFO NCs demonstrate a green fluorescence signal (**Figure 4f**), since Fe^{3+} could consume overexpressed H_2O_2 to yield $\bullet\text{OH}$. Nevertheless, the elevated green fluorescence can be found in BFO NCs with US irradiation group, manifesting that chemodynamic effect produced by BFO NCs with US can obviously improve the generation efficiency of $\bullet\text{OH}$ (**Figure 4e**). To distinguish the dead and living cells more intuitively, HeLa cells were co-stained with propidium

iodide (PI) and Calcein-AM after treated with BFO NCs under acidic or neutral culture conditions for 4 h. The cell morphology was observed by the CLSM (**Figure 4f**). The images demonstrate that no apparent or only minor fraction of HeLa cells was injured by H₂O₂, US and BFO NCs in both neutral and acidic microenvironments, separately. Reasonably, when HeLa cells were cultured with BFO NCs in the presence of US, almost all cells were dead under acidic condition, illuminating that US-enhanced CDT had apparent anticancer activity. The flow cytometry experiments confirmed that the tumor cells were effectively killed by BFO NCs with US irradiation (**Figure S20**). Thus, BFO NCs have high chemodynamic toxicity against tumor cells, which could act as an US-enhanced CDT agent for efficient treatment of malignant tumors.

To obtain more precise and significant anticancer therapeutic effect, imaging guidance is beneficial to achieve accurate US concentrating on the tumor when excited with sound source, while decreasing a possible damage to surrounding normal tissues. Based on the intrinsic fluorescence property of as-prepared nanoparticles, *In Vivo* fluorescent imaging of BFO NCs was provided. The BFO NCs were introduced into the abdomen of the mouse by subcutaneous injection, and imaged using an *In Vivo* fluorescence imaging device. The imaging was based on the 1250 ± 20 nm down-conversion luminescent signals of BFO NCs in the abdomen regions when irradiated upon 808 nm laser (200 mW/cm²). The significant increment of fluorescence signals in the abdomen of the mouse was detected after injection, suggesting that the BFO NCs can serve as a fluorescence imaging tool for tracing the theranostic process (**Figure S21**).

As we known, CT as the most convenient diagnostic means has been widely applied in clinic due to the inherent advantages such as deep tissue penetration depth, and high spatial and temporal resolution. Aside from the fluorescence imaging, the BFO NCs could also be utilized for CT imaging based on intensive X-ray attenuation property of the Bi component. Upon increasing the concentration of BFO NCs, the CT signal value was gradually enhanced, and the Hounsfield Unit (HU) slope was evaluated to be 16.33 HU g L⁻¹ (**Figure 5a,b**). As a contrast, the *In Vitro* CT imaging effect of clinical CT imaging contrast agent iohexol was provided. *In Vitro* CT signal gradually increased and the straight slope of iohexol (14.29 ± 0.43) was lower than that of BFO NCs at the corresponding concentration (**Figure S22**). The *In Vivo* CT imaging on mice having the treatment of BFO NCs illustrated a significant contrast effect in the tumor location, and the signal intensity increased by 21.3-fold in comparison with the control group (**Figure 5c,d**). The cross-sectional CT line profiles before and after 24 h administration of BFO NCs are

shown in **Figure 5e,f**. As expected, the CT contrast effect of BFO NCs allows the tracking of drug delivery and the monitoring of the treatment process *In Vivo*.

Figure 5. (a) CT images of BFO NCs in aqueous solution with varied concentrations. (b) CT values of BFO NCs under different concentrations. *In Vivo* CT images of tumor-bearing mice (c) at pre-injection and (d) at 24 h post-injection of BFO NCs, and corresponding cross-sectional CT line profiles (e) before and (f) after the intravenous injection. (g) Longitudinal and (h) transverse

relaxation rates of BFO NCs and H₂O₂-responded BFO NCs (pH = 6.0). Inset: corresponding MRI photographs. (i) Intensity changes of T_1 -weighted and T_2 -weighted MRI signals at varied times, and (j) corresponding magnetic resonance images of mice *In Vivo* (red dotted circles), indicating the H₂O₂-enhanced MRI property.

Additionally, the special multiferroic endows the BFO NCs with a potential contrast effect for MRI, which is beneficial to orient tumor theranostics. It is different from the previously reported constant MRI, the *In Vitro* experimental results revealed a significant signal change for T_1/T_2 -weighted MRI. Before reacting with H₂O₂, the longitudinal relaxivity (r_1) and transverse relaxivity (r_2) values were evaluated to be 0.132 and 1.126 mM⁻¹ s⁻¹ separately, resulting in a specific r_2/r_1 value of 8.507. After BFO NCs interact with H₂O₂, apparent r_1 ($r_1 = 0.312$ mM⁻¹ s⁻¹) and r_2 ($r_2 = 6.750$ mM⁻¹ s⁻¹) value increase was detected, and thus a higher r_2/r_1 ratio of 21.634 was obtained (**Figure 5g,h**). To further affirm the *In Vivo* MRI performance, the BFO NCs were administrated into tumor-bearing mice intravenously, and imaging effect was performed and monitored before and after injection at various time points. As the injection time prolonged, the T_2 -weight negative MRI signal gradually increased, which was ascribed to the enhanced permeability and retention (EPR) effect for effective accumulation of BFO NCs in tumor site to interact with endogenous H₂O₂ namely Fenton-like reaction, as indicated by the tumor region in the images (**Figure 5i,j**). In accordance with the *In Vitro* detection, the above experimental results also indicate that the multiferroic BFO NCs are a significant generation of contrast agent, presenting a feasibility for the implementation of self-enhanced MRI contrast. The intriguing MRI property of BFO NCs exhibits a prospect for the imaging-guided and monitored cancer therapeutics.

Pharmacokinetics of administrated BFO NCs was evaluated to assess the body clearance property of the nanomaterials. Time-dependent biodistribution of the nanocatalysts in major organs and tumor was carried out by measuring the content of BFO NCs, as revealed in **Figure 6a**. Noticeable retention of BFO NCs was primarily distributed in spleen and liver of the treated mice because of the capture by the reticuloendothelial system, which is a general observation in nanomedicine. Relative accumulation amounts of BFO NCs in the tumor site are 5.01% at 2 h post-injection, 7.12% after 24 h administration, and ultimately 6.98% at 48 h post-injection, responsible for higher therapeutic efficacy. Effective accumulation of BFO NCs at tumor should

be due to the EPR effect. Based on blood circulation experiments, the plasma terminal half-life of BFO NCs was estimated to be 2.98 h (**Figure 6b**). As demonstrated in **Figure 6c**, the ablating rate coefficients of BFO NCs were counted to be $-0.33 \mu\text{g mL}^{-1} \text{h}^{-1}$ in the first phase, which declined to $-0.02 \mu\text{g mL}^{-1} \text{h}^{-1}$ in the second stage at transition time point of 2.42 h. Distinct volume percentage of accumulation (**Figure 6d**) for BFO NCs demonstrates arising distribution kinematics during the blood circulation systemically.

Figure 6. Pathology and pharmacokinetics results of BFO. (a) Biodistribution of Bi (injected dosage ID) of equivalent Bi per gram of tissues) in tumor and major tissues (kidney, heart, spleen, liver, and lung) at 2, 24, and 48 h of intravenously injected BFO ($n = 3$). (b) Curve of blood circulation upon intravenous injection of BFO ($n = 5$). The half-time ($t_{1/2}$) is 2.98 h. (c) Eliminating rate curve obtained from the curve of blood circulation based on $\ln(\text{concentration})$ - time relation, showing a two-stage elimination rate of injected BFO. (d) Volume percentage of distribution (Vd%) for intravenously injected BFO NCs.

Before executing *In Vivo* anticancer therapy, the hemocompatibility of the BFO NCs was further investigated. The hemolysis experiment results demonstrated no significant hemolytic effect (less than 2%) from the BFO NCs even with the concentration of up to 800 $\mu\text{g mL}^{-1}$ in PBS, implying its good hemocompatibility (**Figure S23**). Firstly, the biosafety *In Vivo* was assessed on tumor-bearing mice intravenously injected with saline (control group) and BFO at various dosages (5, 10, and 20 mg kg^{-1}). The mouse body weight was measured in experimental periods to research whether the BFO NCs have systemic toxicity. All the injection groups of BFO NCs revealed no evident weight change in comparison to the initial weight (**Figure S24a**). Subsequently, blood routine analysis and blood biochemistry *In Vivo* were comprehensively implemented to research the long-term toxicity of BFO NCs. Blood was acquired from healthy mice from tail vein under various time points after administration of BFO NCs intravenously. All hepatic and kidney function indexes, containing alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine (CREA) were evaluated. The levels of aminotransferase under the three dosages were basically consistent with the control group (**Figure S24b-d**), suggesting compatible kidney and hepatic functions of BFO NCs. Routine indexes of blood analysis including hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), red blood cells (RBC), and mean corpuscular volume (MCV) were determined as shown in **Figure S24e-i**. All the indicators fall into normal ranges, indicating no distinct biotoxicity of BFO NCs at different injection dosages *In Vivo*. Histological analysis on some major organ tissues (*i.e.*, heart, kidney, liver, lung, and spleen) from mice sacrificed after the treatment of 14 days with BFO NCs through tail vein administration was also conducted (**Figure S25**). No observable tissue damage or inflammatory lesion was detected, indicating high histocompatibility of BFO NCs. Such experimental results reveal that BFO NCs have an insignificant systemic toxicity, strengthening its biomedical utilization as a high-efficiency theranostic nanoplatform.

Figure 7. (a) Schematic illustration for establishing U14 tumor xenograft, administration modality of saline and BFO NCs, and tumor suppression. (b) Mouse body weights after treating with saline (control group) and BFO NCs (therapeutic groups) having concentrations of 5 and 10 mg kg⁻¹ intratumorally and intravenously. Relative tumor volumes of mice collected every 2 days after treating with saline and BFO NCs by (c) intravenous and (d) intratumoral administration (n = 5). (e) Tumor inhibition rates and volumes of dissected tumors from control and therapeutic groups after the 14-day treatment. Photographs of dissected tumors for (f) intravenous and (g) intratumoral therapeutic groups after the 14-day treatment. (h) Histopathology images of

dissected tumor xenografts stained with TUNEL and H&E staining assay. Scale bar: 100 μm . * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Encouraged by the *In Vitro* anticancer effects of BFO NCs, we then assessed the *In Vivo* imaging performance and treatment efficiency by establishing U14 tumor-bearing mice. After one week, the mice were divided randomly to control group (injection of saline) and therapeutic groups (injection of BFO NCs with concentrations of 5 and 10 mg kg^{-1}), and treated intratumorally and intravenously to research treatment efficiency, respectively (**Figure 7a**). In the course of 14 days of treatment, no apparent change in mice body weights was detected for control and all treatment groups, confirming that no prominent toxicity was triggered by the administration of BFO NCs (**Figure 7b**). According to the relative tumor volume growth curves, these treatment modalities provide satisfactory tumor growth inhibition effect, as indicated in **Figure 7c,d**. More specifically, in accordance with the changes of relative tumor volumes, the inhibition rates of tumor growth were assessed to be 56.1% and 83.8% at the dosages of 5 and 10 mg kg^{-1} injected intravenously, as well as 44.9% and 73.5% at the same dosages injected intratumorally (**Figure 7e**). After the treatment process, the tumors of representative mice from different treatment groups were excised and presented in **Figure 7f,g**, which directly exhibit that the tumor growth was validly inhibited by using BFO NCs intratumorally or intravenously. The apparent damage of cancer cells was immediately detected in hematoxylin and eosin (H&E) staining images of tumor tissues. Significantly promoted cell apoptosis was also found in TdT-mediated dUTP nick-end labeling (TUNEL) images of cancer cells (**Figure 7i**). To further confirm the US-enhanced anticancer treatment effect of BFO NCs, the tumor-bearing mice were administrated with saline, US (3 W/cm^2 , 40 kHz, 5 min, 50% duty cycle), BFO NCs only, and BFO NCs with US irradiation groups (**Figure 8a**). The tumors in only BFO NCs group were smaller than those in control groups (saline and US), and the tumor growth inhibition rate was up to 41.2% (**Figure 8b,d**), showing that CDT has a specific therapeutic efficacy. In addition to CDT, the tumors of mice were also exposed to the US treatment. As compared with US alone or CDT group, the relative tumor volume in BFO NCs with US irradiation group apparently decreased and the tumor growth suppression rate was estimated to be 82.8%, indicating that therapeutic performance of CDT can be effectively improved by US. In addition, there was no evident change of the mouse body weight (**Figure 8c**). The excised tumors and representative mice from

these treatment groups were also presented (**Figure 8e**). The TUNEL and H&E staining of the dissected tumors in diverse groups were performed to assess the pathological damage, as revealed in **Figure 8f**. In accordance with the tumor growth suppression results, the BFO NCs with US irradiation group exhibited large hypocellular and necrotic areas in the tumor tissue. However, no pathological change could be detected in major organs in control groups (**Figure S26** and **S27**), confirming that the as-synthesized nanomaterials possess favorable biocompatibility and biosafety.

Figure 8. *In Vivo* anticancer therapy performance with BFO NCs. (a) Schematic illustration of US-enhanced CDT process on mice *In Vivo*. (b) Tumor volume growth curves of tumor-bearing mice after various treatments. (c) Body weight changes of tumor-bearing mice in the groups during 14-day treatment period. Error bars represent standard deviations ($n = 5$). (d) Inhibition rates and volumes of dissected tumors from treatment groups after 14-day therapy. (e) Photographs of dissected tumor tissues from treatment groups after 14-day therapy, and representative tumor-bearing mice in each group. (f) Histopathology images of dissected tumor xenografts stained with the TUNEL and H&E assay. Scale bar: 100 μm . (g) Schematic therapeutic mechanism of BFO NCs and hydroxyl radical-mediated tumor cell death. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Theoretically, tumor cells promote essential oxidative stresses by appropriately increasing the content of ROS, and this process plays an important role in the cell growth and multiplication.

With the overproduction of superoxide dismutase, the superoxide anion radicals are transformed into a great deal of H_2O_2 . The BFO NCs with moderate particle size facilitate their transportation in the tumor blood vessel and accumulation in the tumor site. Then, Fe-based BFO NCs participate in the Fenton-like reaction utilizing the overexpressed H_2O_2 under the mild acidic TME to yield a plentiful of toxic free radicals. Intriguingly, assisted by external US, the production of $\bullet\text{OH}$ free radical is significantly enhanced, which can be attributed to the interaction of US with generated cavitation bubbles by powerful shockwave, triggering local violent turbulence and expediting the mass transfer rate of BFO NCs. Because of the $\bullet\text{OH}$ free radical generation, the cancer cell apoptosis occurs, inducing tumor cell death through hydroxyl radical-mediated oxidative damage pathway. Subsequently, the BFO NCs would enable instant excretion from the body after operating the anticancer treatment efficiency (**Figure 8g**).

CONCLUSION

In a word, we have successfully presented that perovskite-type mixed oxide BFO NCs can serve as “all-in-one” theranostic nanocatalysts, which catalyze endogenous H_2O_2 disintegration assisted with US for effectively improving toxic free radical production to acquire multimodality imaging-guided US-enhanced CDT. The BFO NCs exhibit satisfactory colloidal stability and biocompatibility. As identified by several kinds of measurements, BFO NCs demonstrate much more efficient US-induced free radical production, since the cavitation bubbles produced by US could trigger partial grievous turbulence and heighten the transfer rate of the Fenton reagents. Interestingly, the special magnetic and optical properties of BFO NCs not only permit CT, MRI and fluorescent imaging, but also prominently enhance the treatment efficacy of the CDT assisted with an external US. At the *In Vivo* level, such US-enhanced CDT with BFO NCs effectually inhibits the tumor growth. The imaging-guided US-enhanced cancer treatment by bimetallic oxide BFO NCs may lead to a noninvasive, convenient and cost-effective approach for future biomedical applications.

EXPERIMENTAL PROCEDURES

Hydroxyl Radical Generation Assessment of BFO NCs. In a typical process, H_2O_2 with different concentration (12.5, 25, 50, and 100 mM) was added into the mixture solution including BFO (200 $\mu\text{g}/\text{mL}$) and 3,3',5,5'-tetramethylbenzidine (0.8 mM), respectively. Comparatively, the

effect of ultrasonic time (15, 30, 60, and 120 s) on the generation of $\bullet\text{OH}$ was investigated at a H_2O_2 concentration of 50 mM. The absorbance at 653 nm wavelength was measured by the UV-vis spectroscopy instrument. The Michaelis-Menten constant (K_M) and maximal velocity ($V_{\max}$) were estimated through the Lineweaver-Burk plot.

Cellular Uptake of BFO NCs. To research the cell internalization of the resulted BFO NCs, the surface of nanocatalysts was embellished with FITC. Appropriate quantities of FITC and (3-aminopropyl)triethoxysilane (APTES) were treated in EtOH (5 mL), and the APTES-FITC conjugate was acquired after magnetic stirring for 12 h. Then, the above solution (10 μL) was introduced to the resulted nanocatalysts under stirring for another 12 h. FITC-labeled BFO NCs were obtained *via* centrifugation. The above experiments were carried out in dark conditions. HeLa cells were planted in a 6-well plate (7000 cells/well) overnight. FITC-labelled BFO NCs (200 $\mu\text{g/mL}$) were incubated with cells for 0.5 and 3 h, separately. After the incubation, the tumor cells were stained with 4',6-diamidino-2-phenylindole for 10 min, rinsed with PBS mildly for twice times, and further immobilized with glutaraldehyde. At last, the cells were observed by CLSM.

Cytotoxicity Measurement of BFO NCs. L929 cells were implanted in a 96-well plate for 12 h, following by incubation with various concentrations of BFO NCs in culture medium solutions (0, 12.5, 25, 50, 100, 200, 400, and 800 $\mu\text{g/mL}$) for various incubation times (24 h and 48 h), respectively. The cell survival rate was monitored by the standard MTT assay. As a comparison, the cell viabilities of the HeLa cells treated with BFO NCs and H_2O_2 having varied concentrations (2.5, 5, 10, 25, 50, 100, and 200 μM) and irradiated upon US at various power density (0, 0.5, 1, 1.5, 2, 2.5, and 3 W/cm^2) were also performed, respectively.

***In Vitro* CDT and US-Enhanced CDT.** HeLa cells were implanted into a 96-well plate for 12 h to grow against the wall. The BFO culture medium with various concentrations was introduced to each well containing H_2O_2 with varied concentrations (50 and 100 μM), and the diluted HCl was used to adjust the pH of the culture medium to 7.4 and 6.0, respectively. After rinsing several times with PBS and adding culture medium (150 μL), the cells were exposed to US (3 W/cm^2 , 40 kHz, 120 seconds). The cell cytotoxicity tests were assessed by a standard MTT assay. After 24 h incubating, MTT solution (20 $\mu\text{g/mL}$) was appended to each well with continuous incubation for 4 h. After that, DMSO (150 μL) was added to each well, and the absorbance ratio was estimated to calculate the cell viability. Furthermore, in order to visualize

the cytotoxicity performances, the HeLa cells were implanted into a 6-well plate and cultured with H₂O₂ and BFO NCs (200 µg/mL) for 24 h. The pH value was modulated using diluted HCl. The cells were treated with US irradiation (3 W/cm², 40 kHz, 120 seconds). Subsequently, the culture medium was thrown away and the cells were rinsed with PBS before staining with Calcein-AM/PI for 30 min. Ultimately, the CLSM was adopted to observe the cellular morphology.

Detection of Intracellular Hydroxyl Radical. HeLa cells were seeded into a 6-well plate and cultured with H₂O₂ and BFO NCs (200 µg/mL) at pH of 7.4 and 6.0 for 6 h, respectively. Then, the cells were exposed to US for 120 seconds (3 W/cm², 40 kHz). Subsequently, non-fluorescence DCFH-DA (25 µM) was appended and co-incubated with the cells for another 30 min. The levels of generated intracellular •OH were assessed *via* detected the fluorescence signal intensity of DCF *via* CLSM.

***In Vivo* Blood Circulation Half-Life of BFO NCs.** The tumor-bearing mice were intravenously treated with BFO NCs. At different injection times (2, 8, 15, and 30 min as well as 1, 2, 4, 8, and 24 h), the blood sample was collected and measured. The concentrations of Bi and Fe (C_p) were further calculated through ICP-MS. Then, the blood circulation half-life ($\tau_{1/2}$) of BFO was estimated.

***In Vivo* Anticancer Therapeutic Performance of BFO NCs.** The tumor-bearing mice were divided into three groups randomly (n = 5). Firstly, the tumors were acquired by subcutaneous injection of U14 cells in the left axilla of mice. Treatment began when the tumors grew to approximately 60 mm³. During the therapeutic periods, BFO NCs with the concentration of 5 and 10 mg kg⁻¹ were introduced into the mice by intratumorally (i.t.) and intravenously (i.v.), respectively. For US-enhanced CDT, the experiments were separated into four groups: (1) PBS; (2) Only US (3 W/cm², 40 kHz, 5 min, 50% duty cycle); (3) BFO NCs; (4) BFO NCs + US (3 W/cm², 40 kHz, 5 min, 50% duty cycle). The mice were treated with BFO NCs at a dose of 10 mg kg⁻¹ during the treatment process. The mouse body weight and tumor size were recorded every two days in the course of treatment. The relative tumor volume was defined as $V_r = V/V_0 \times 100\%$ (V_0 represents the tumor volume before the treatment). The second injection of BFO NCs was performed at the fourth day after the first treatment. After 14-day treatment, all the mice were dissected, and the tumors and main organs from these treatment groups were harvested for further histopathological analysis. The pathologic analysis was observed by CLSM.

CT Imaging Performance *In Vitro* and *In Vivo*. The CT imaging tests *In Vitro* were carried out using a 64-slice clinical CT instrument. The BFO NCs were dissolved in PBS with varied concentrations of 0, 1, 2, 4, 8, and 16 mg/mL, and then put in tubes for the imaging. To execute the CT imaging effect of BFO NCs *In Vivo*, mice were first anesthetized by injecting an anesthetic into their abdominal cavity, and then BFO NCs (10 mg kg⁻¹) were injected to the mice intravenously. The CT imaging performance was evaluated before and after the injection. The CT signal values were acquired by CT scanner software.

ASSOCIATED CONTENT

Supporting Information Available: This material is available free of charge *via* the Internet at <http://pubs.acs.org>.

Synthesis and characterization details, TEM images, PXRD patterns, FTIR spectra, Raman spectra, XPS spectra, field-dependent magnetization curves, UV–vis spectra, dynamic light scattering, Zeta potential, thermogravimetry analysis, photothermal performance, bismuth ion energy levels, chemodynamic performance, cell viability, Bio-TEM images, flow cytometry data, fluorescence imaging, *In Vitro* CT images of iohexol, hemolysis of red blood cells, kidney and liver function indexes, and histopathology images.

The authors declare no competing financial interest.

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ACKNOWLEDGMENTS

The work was financially supported by the National Natural Science Foundation of China (51972075, 51772059, and 51972076), the Natural Science Foundation of Shandong Province (ZR2019ZD29), the Heilongjiang Province Postdoctoral Foundation (LBH-Z19129), and the Fundamental Research Funds for the Central Universities. The work was also supported by the Singapore National Research Foundation Investigatorship (NRF-NRFI2018-03).

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