



Albumin-mediated synthesis of fluoroperovskite KMnF_3 nanocrystals for T_1 - T_2 dual-modal magnetic resonance imaging of brain gliomas with improved sensitivity

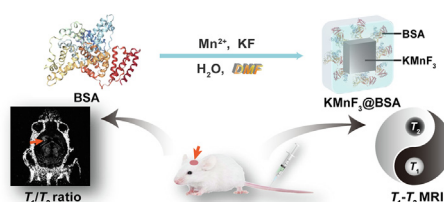
Xuechun Wang, Yuping Hu, Rui Wang, Peng Zhao, Wei Gu, Ling Ye*

School of Pharmaceutical Sciences, Capital Medical University, Beijing 100069, PR China

HIGHLIGHTS

- A “one-pot” biomimetic method was developed to prepare KMnF_3 nanocrystals.
- The KMnF_3 nanocrystals can act as T_1 - T_2 dual-modal contrast agents for brain glioma imaging.
- Postprocessing of concurrently obtained T_1 - T_2 MRI images leads to an improved sensitivity.
- The developed biomimetic method is a general approach to prepare complex metal fluorides.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

Biomimetalization
Magnetic resonance imaging
 T_1 - T_2 dual-modal contrast agents
 KMnF_3 nanocrystals
Brain gliomas

ABSTRACT

Sensitivity of magnetic resonance imaging (MRI) enhanced by T_1 or T_2 contrast agents remains an unmet medical need for accurate diagnosis of gliomas with infiltrative nature. Herein, we report the synthesis of bovine serum albumin-mediated KMnF_3 nanocrystals (KMnF_3 @BSA NCs) via a simple “one-pot” biomimetic method. The KMnF_3 @BSA NCs composed of one paramagnetic component have high relaxivities ($r_1 = 6.14 \text{ mM}^{-1}\text{s}^{-1}$, $r_2 = 149.69 \text{ mM}^{-1}\text{s}^{-1}$) and provide complementary T_1 - T_2 dual-modal MRI images with the same in-plane geometries in glioma-bearing mice on a 7.0 T MRI scanner. Furthermore, algebra algorithm processing of the T_1 and T_2 images suppresses the ambiguity and greatly increases the contrast between tumors and the surrounding tissue, leading to an improved sensitivity. The biomimetic prepared KMnF_3 @BSA NCs thus have great potential in accurate MRI detection of gliomas. Moreover, this simple biomimetic method could be applied to prepare other complex metal fluorides.

1. Introduction

Magnetic resonance imaging (MRI), because of its noninvasive characteristics and exquisite soft tissue contrast with high spatial-temporal resolution, is one of the most powerful and indispensable imaging tools for clinical tumor diagnosis and preoperative delineation [1,2]. MRI contrast agents are commonly used to enhance the visibility of the biological target from healthy tissue [3]. Depending on the magnetic

susceptibility, contrast agents can be classified as positive (T_1) or negative (T_2), which respectively change the longitudinal and transverse relaxation times of protons and result in brighter (T_1) and darker (T_2) contrast enhancements [4]. Specifically, T_1 contrast agents facilitate high resolution between tissues [5], whereas T_2 contrast agents facilitate a high feasibility for detection of a lesion [6]. However, MRI enhanced by either T_1 or T_2 contrast agents may still suffer from possible artifacts originating from certain endogenous factors, such as

* Corresponding author.

E-mail address: lingye@ccmu.edu.cn (L. Ye).

<https://doi.org/10.1016/j.cej.2020.125066>

Received 8 January 2020; Received in revised form 21 March 2020; Accepted 12 April 2020

Available online 15 April 2020

1385-8947/ © 2020 Elsevier B.V. All rights reserved.

hemorrhage, calcification, and metal deposits [7]. For accurate diagnoses and precise delineations of tumors, artifact-free MRI is highly desirable but remains challenging.

Recently, the T_1 - T_2 dual-modal strategy, which provides complementary diagnostic information in one instrument with the same penetration depth and spatial/time resolutions almost simultaneously, has been proposed to suppress the ambiguities and make possible self-confirmed MRI signals [8]. In this scenario, T_1 - T_2 dual-modal contrast agents are essential. Generally, T_1 - T_2 dual-modal contrast agents are formulated either by doping a T_1 component (e.g., Gd^{3+} and Mn^{2+} ions) into a T_2 matrix (e.g., super-paramagnetic iron oxide) [9,10] or by integrating T_1 and T_2 contrast agents into a hybrid platform [11,12]. However, interference between two neighboring T_1 and T_2 components is inevitable, leading to an unwanted quenching effect of both T_1 and T_2 signals when using MRI [13,14]. To eliminate this interference, the T_1 - T_2 contrast agents composed of one single component are preferred [15].

Although manganese-based contrast agents are usually used as T_1 contrast agents [16,17], they have recently attracted renewed interest because of their ability to serve as T_1 - T_2 dual-modal contrast agents through careful chemical design and engineering [18]. For instance, the SiO_2 -coated and hollow manganese oxide nanoparticles (NPs) have been synthesized and used in T_1 - T_2 dual-modal MRI [19,20]. Apart from manganese oxide NPs, $KMnF_3$ NPs were reported as a novel T_1 contrast agent with a relatively high r_1 relaxivity [21]. To ensure the designed morphology and crystallinity of $KMnF_3$ NPs, high temperature, long reaction time, and complicated surface-modified process are typically involved in the reported methods [21–26]. Alternatively, biomimetic approach has been shown to be a green and highly efficient way to prepare NPs under ambient conditions [27]. In particular, albumins have been used because of their excellent biocompatibility and facile surface conjugation [28]. In addition, it has been reported that the complexes of albumin with paramagnetic materials enhance T_2 relaxation properties due to the intermolecular field coupling effect [8], which is especially beneficial for obtaining T_1 - T_2 dual-modal MRI contrast agents to improve the sensitivity of MRI. However, the applicable of albumin-mediated biomimetic way to fabricate crystalline $KMnF_3$ NPs and the potential of albumin-templated $KMnF_3$ as T_1 - T_2 dual-modal contrast agents has not been adequately explored.

In the present study, we reported a “one-pot” biomimetic method to prepare the bovine serum albumin-mediated $KMnF_3$ nanocrystals ($KMnF_3$ @BSA NCs) as illustrated in Scheme 1. Specifically, BSA was used as the template to fabricate $KMnF_3$ and more importantly, it helped to improve the water dispersibility, biocompatibility, and relaxation properties of $KMnF_3$. As such, the $KMnF_3$ @BSA NCs composed of one paramagnetic component exhibited unique high r_1 and r_2 relaxivities, which ensured simultaneous T_1 - T_2 dual-modal MRI of brain gliomas. By postprocessing the concurrently obtained T_1 and T_2 MRI images, an improvement in MRI sensitivity was achieved. In addition,

we examined the feasibility of this simple biomimetic method to prepare other complex metal fluorides such as $KCoF_3$, $KGdF_4$, and $KEuF_4$.

2. Experimental

2.1. Synthesis of $KMnF_3$ @BSA NCs

$KMnF_3$ @BSA NCs were prepared via a “one-pot” biomimetic procedure at room temperature. In a typical process, 250 mg of BSA was dissolved in 8 mL of deionized water with vigorous stirring. Then, 1 mL of $MnCl_2 \cdot 4H_2O$ (1.87 mM) was added. After stirring for 30 min, 1 mL of $KF \cdot 2H_2O$ (18.7 mM) was quickly added. It was observed that the transparent solution gradually turned into white, indicating the formation of $KMnF_3$. After 15 min of reaction, 5 mL of N, N-dimethylformamide (DMF) was added to terminate the reaction. Fifteen minutes later, the products were separated by centrifugation (6000 rpm, 5 min), washed with deionized water and DMF for three times, and lyophilized to yield the $KMnF_3$ @BSA NCs. A variety of complex metal fluoride compounds, such as $KCoF_3$ @BSA, $KGdF_4$ @BSA, and $KEuF_4$ @BSA, were synthesized with their corresponding chloride following the same “one-pot” biomimetic procedure.

2.2. Relaxivity measurement

For relaxivity measurement, the $KMnF_3$ @BSA NCs were dispersed in deionized water with various Mn concentrations (0, 0.025, 0.05, 0.1, 0.2, 0.4 mM) and the relaxation times were obtained on a 7.0 T MRI scanner (Bruker Pharmascan, Germany) by the RARE- T_1 + T_2 -map sequence using the previous reported parameters [29] with minor modification in multiple echo times (TE): TE = 11.00, 33.00, 55.00, 77.00, 99.00 ms. The calculation of the relaxivity values were via the slope of inverse relaxation times ($1/T$) versus Mn concentration.

2.3. In vivo MRI

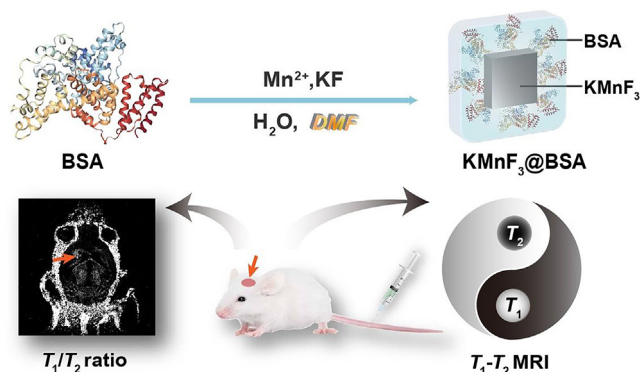
The procedures of animal experiments strictly followed the standard protocols approved by the ethical committee of Capital Medical University. The glioma-bearing mice ($n = 3$) were anesthetized. After that, 200 μ L of $KMnF_3$ @BSA NCs was intravenously injected into each mouse at a dosage of 5 mg Mn/kg. In vivo T_1 - and T_2 -weighted MRI images were obtained on a 7.0 T MRI scanner (Bruker Pharmascan, Germany) with the multi-slice multi-echo (MSME) and rapid acquisition with relaxation enhancement (RARE) sequences. MRI images were collected at pre- and different time post-injection with the parameters previously reported elsewhere [30] with a slight difference in repetition times (TR)/TE: TR/TE = 300/8.6 ms (T_1), 4000/80 ms (T_2). The T_1/T_2 ratio images were generated according to an algebra algorithm (image division) reported elsewhere [31,32].

3. Results and discussion

3.1. Formation of $KMnF_3$ @BSA NCs

Synthesis of $KMnF_3$ with high crystalline properties mainly relied on thermal decomposition and hydrothermal methods, which required high temperature and/or high pressure. Moreover, a follow-up multi-step modification was needed to render the water dispersibility and functionality [21–24]. Herein, we developed a “one-pot” biomimetic method to synthesize highly water-dispersible $KMnF_3$ @BSA NCs under ambient conditions. In this process, BSA acted as a template to coordinate Mn^{2+} ions, and the subsequent addition of KF triggered the nucleation and growth of $KMnF_3$. Fifteen minutes later, DMF was added and the $KMnF_3$ @BSA NCs with high crystalline structure were finally obtained in another 15 min.

Transmission electron microscopy (TEM) images showed that the as-prepared $KMnF_3$ @BSA NCs had a cubic morphology with the



Scheme 1. Schematic illustration of “one-pot” synthesis of $KMnF_3$ @BSA NCs for T_1 - T_2 dual-modal MRI of brain gliomas with improved sensitivity.

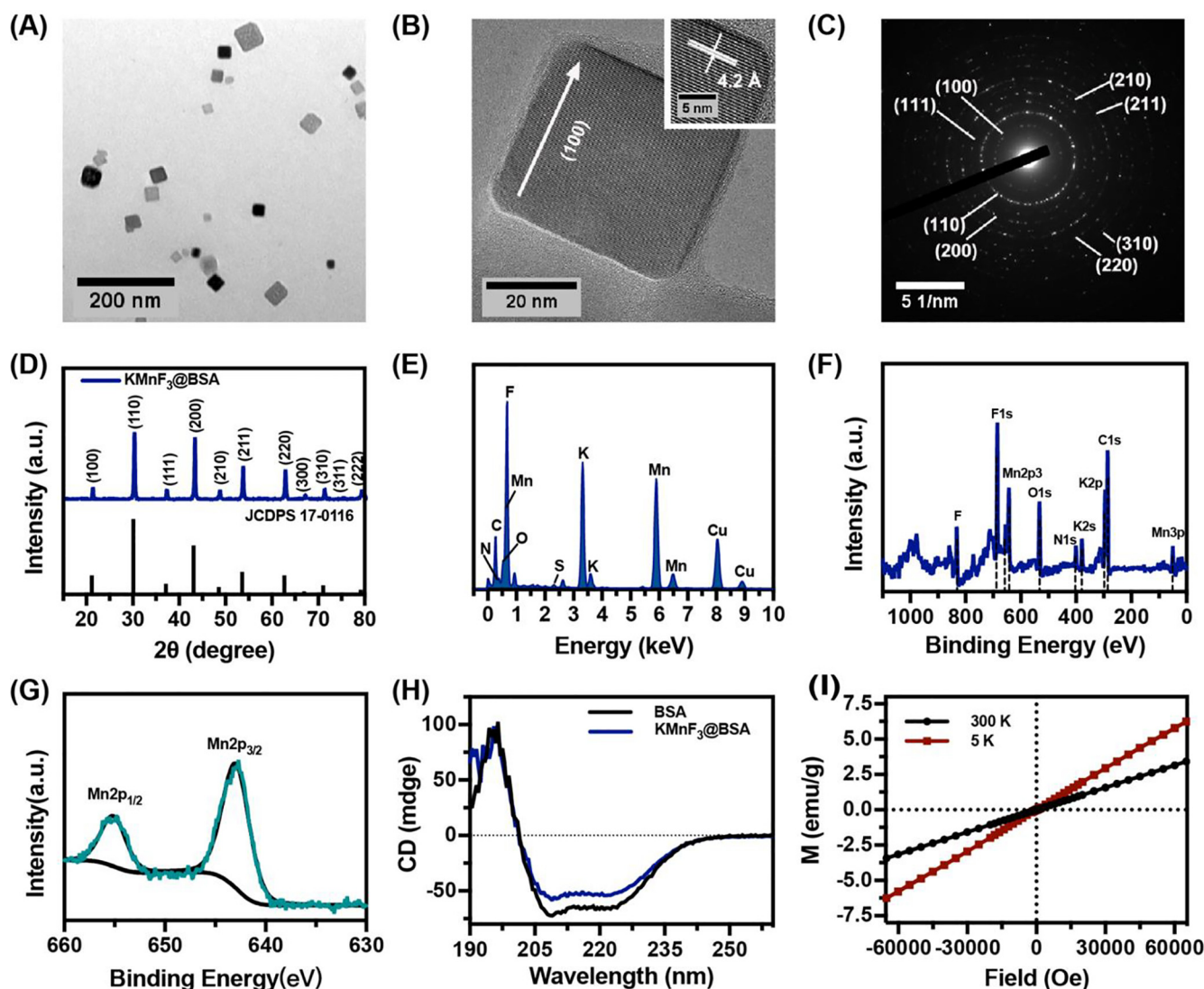


Fig. 1. Characterizations of KMnF_3 @BSA NCs. (A) TEM image, (B) high resolution TEM image, (C) SAED pattern, (D) XRD pattern, (E) EDS spectrum, (F) XPS survey, (G) Mn 2p XPS spectra, (H) CD spectra, and (I) M - H curves.

average size of 39 ± 3.63 nm (obtained from 200 measured particles) (Fig. 1A). The high resolution TEM image (Fig. 1B) indicated a clear lattice spacing of $4.2 \text{ \AA}$, matching with the (1 0 0) lattice planes of KMnF_3 . The crystalline nature was also supported by the crystalline diffraction rings in a selected area of the electron diffraction (SAED) pattern (Fig. 1C). Moreover, the powder X-ray diffraction pattern (XRD) (Fig. 1D) was in good agreement with the cubic perovskite KMnF_3 (JCPDS 17-0116). In addition, energy dispersive X-ray spectrometer analysis (Fig. 1E) of individual nanoparticles verified the presence of Mn, K, and F in an atomic ratio of nearly 1:1.06:2.54, which was close to the stoichiometric ratio of the molecular formula. X-ray photoelectron spectroscopy (XPS) survey spectrum in Fig. 1F shows characteristic peaks of F1s, K2s, Mn2p3, C1s, N1s, and O1s, which revealed the combination of KMnF_3 and BSA. High resolution Mn 2p XPS spectrum in Fig. 1G revealed Mn2p_{3/2} and Mn2p_{1/2} peaks at 654.6 eV and 642.6 eV, respectively, corresponding to the presence of Mn^{2+} in the KMnF_3 NCs. In addition, dynamic light scattering showed a hydrodynamic diameter of about 80 nm (Fig. S1A), which was slightly larger than that measured by TEM images, possibly due to the BSA coating surface of the NCs [33]. No notable aggregation or precipitate was found for KMnF_3 @BSA NCs in deionized water, saline, or fetal bovine serum (FBS) during a 7-day storage period (Fig. S1B), indicating the good stability of the NCs.

The albumin mediated formation of the KMnF_3 was confirmed by Fourier transform infrared (FT-IR) and circular dichroism (CD) spectra. As expected, the well-known characteristic peaks of the albumin template such as the O-H stretching vibration at 3290 cm^{-1} , and amide I and amide II bands at 1640 and 1530 cm^{-1} could be found in the FT-IR spectra of both BSA and KMnF_3 @BSA NCs (Fig. S2), suggesting the successful binding of KMnF_3 to the template albumin. CD spectra are generally used to examine conformational changes in the secondary structures of proteins. Fig. 1H shows that the KMnF_3 NCs induced a slight conformational change in BSA, inferring the growth of BSA mediated KMnF_3 and the negligible effect of DMF on BSA conformation.

The magnetic properties of KMnF_3 @BSA NCs were characterized using a superconducting quantum interference device at 5 K and 300 K. As anticipated, the KMnF_3 @BSA NCs exhibited typical anti-ferromagnetic properties (Fig. 1I), which agreed with previous reports regarding KMnF_3 [21,24]. In addition, according to the inflexion in the temperature-dependent magnetization curves (Fig. S3), the Néel temperature (T_N) was determined to be 84.9 K. It is known that the anti-ferromagnetic order of anti-ferromagnetic materials only exists at sufficiently low temperature, and disappears above the T_N . Hence, similar to other manganese-based nanomaterials, the KMnF_3 @BSA NCs are paramagnetic at ambient conditions are suitable for acting as contrast agents.

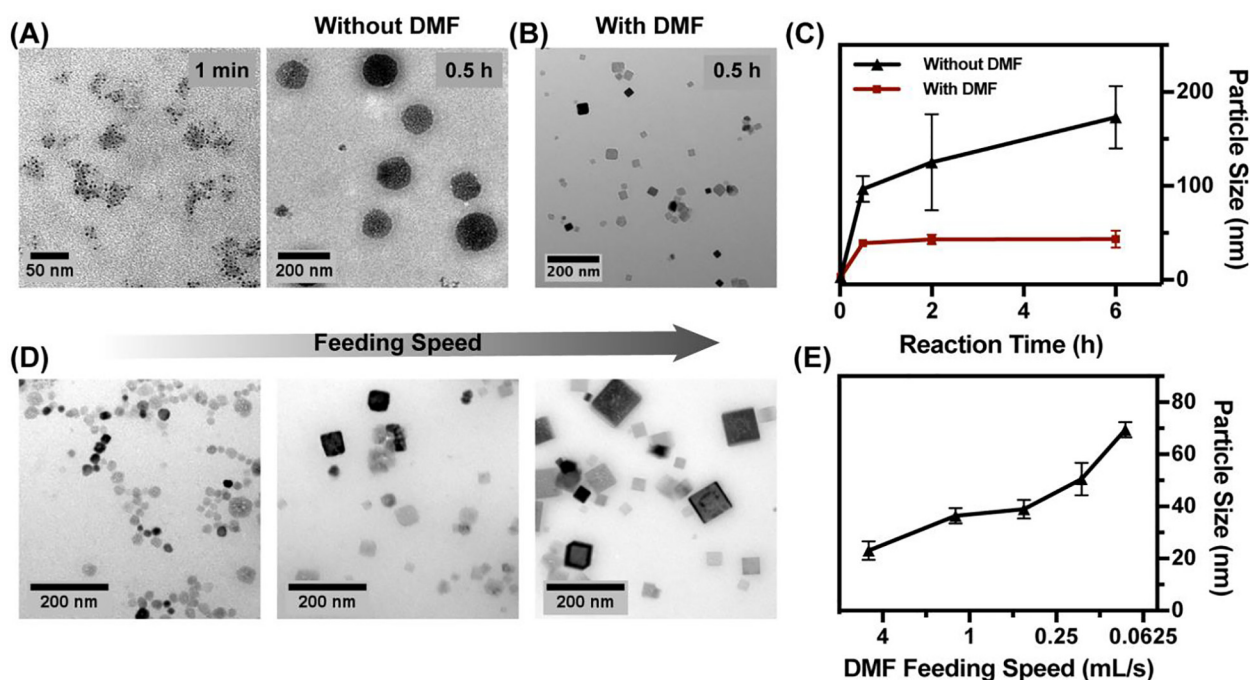


Fig. 2. Formation of KMnF₃@BSA NCs. (A) TEM images of KMnF₃@BSA NCs in the absence of DMF, (B) KMnF₃@BSA NCs prepared in the presence of DMF, (C) size evolution of KMnF₃@BSA NCs prepared in the absence of DMF as function of reaction time, (D) TEM images of KMnF₃@BSA NCs prepared with different DMF feeding speeds, and (E) average size of KMnF₃@BSA NCs prepared with different DMF feeding speeds.

During the preparation of KMnF₃, it was found that the nanoclusters of KMnF₃ were formed at 1 min, and the spherical NPs of KMnF₃ were obtained with the average size of about 100 nm (Fig. 2A) thirty minutes later. Moreover, with the increasing of reaction time, the size of KMnF₃ kept increasing (Fig. 2C), indicating that the formation of KMnF₃ was fast and uncontrollable. However, it is worthwhile to note that the proper particles size for medical applications should be < 100 nm [34–36]. Therefore, a timely termination of the growth of KMnF₃ is demanded. It is reported that an appropriate non-aqueous medium (e.g. alcohol) could stabilize the size of fluorides [37]. Therefore, we attempted to introduce organic solvent into the biomimetic reaction system to control the growth of KMnF₃. After screening of a number of potential non-aqueous solvents (e.g. ethanol, acetone, DMF, and DMSO), we found that addition of DMF could effectively cease the reaction without notable BSA denaturation. Encouraged by this, the effect of DMF on KMnF₃@BSA growth was further examined. As presented in Fig. 2B, addition of DMF led to the formation of smaller sized particles around 40 nm, which was independent of the reaction time (Fig. 2C). Interestingly, a notable morphologic change from sphere to cube was evidenced. Further studies showed that the increasing feeding rate of DMF also resulted in smaller particle sizes (Fig. 2D and E). Thus, DMF was one of the key factors in the biomimetic formation of KMnF₃@BSA NCs with desired size and morphology. Although further investigation is needed to elucidate the underlying mechanism of DMF, it is likely that the formation of cubic KMnF₃@BSA NCs is due to the specific adsorption of DMF on the surface of crystal nuclei, which not only altered the reaction conditions (e.g. polarity) but also affected the crystallization rate and mechanisms in the biomimetic process [37–40].

Complex metal fluorides have been intensively investigated as bioactive probes for magnetic resonance and luminescence in cancer detection [41,42]. Beside KMnF₃, several complex metal fluorides including KCoF₃, KGdF₄, and KEuF₄ were synthesized by the biomimetic method to demonstrate the generality of this approach. TEM images of KCoF₃@BSA, KGdF₄@BSA, and KEuF₄@BSA prepared without DMF are presented in Fig. 3A–C. As can be seen, the NPs exhibited an aggregated state with diameters of 79.83 ± 11.71 , 74.54 ± 3.93 , and 44.67 ± 5.12 nm, respectively. Their corresponding XRD patterns are

presented in Fig. S4B–D, which showed a cubic perovskite phase of KCoF₃ (JCPDS 18-1006), and a pure cubic phase of KGdF₄ and KEuF₄ (CaF₂ type, space group: Fm-3m) [41]. In contrast, when DMF was involved in the reaction, the KCoF₃@BSA, KGdF₄@BSA, and KEuF₄@BSA appeared to be more dispersible with smaller sizes of 26.52 ± 2.08 , 19.22 ± 1.75 , and 22.45 ± 2.62 nm, respectively (Fig. 3D–F). In addition, their XRD patterns in Fig. 3G–I showed no obvious difference with NPs prepared without DMF, indicating the successful preparation of the KCoF₃@BSA, KGdF₄@BSA, and KEuF₄@BSA NCs. The regulatory role of DMF in this biomimetic process has again been proven and this simple “one-pot” method has the versatility to prepare nano-sized, water-dispersible complex metal fluorides with high crystallinity in ambient conditions, which will facilitate the application of these nanomaterials in a broad spectrum of fields.

3.2. Relaxation properties

To evaluate the potential of KMnF₃@BSA NCs as MRI contrast agents, magnetic relaxation property measurements were performed on a 7.0 T MRI scanner (Fig. 4). The r_1 and r_2 relaxivities calculated via the slopes of inverse relaxation times ($1/T$) versus Mn concentration plots were 6.14 and $149.68 \text{ mM}^{-1}\text{s}^{-1}$, respectively. Both of the relaxivity values were considerably higher than those of the MnO₂@BSA NPs using the similar BSA-templated biomimetic method ($1.60 \text{ mM}^{-1}\text{s}^{-1}$ for r_1 and $19.09 \text{ mM}^{-1}\text{s}^{-1}$ for r_2) (Fig. S5). The higher r_1 relaxivity of KMnF₃@BSA compared to that of MnO₂@BSA could be ascribed to the covalent-ionic behavior of KMnF₃ [43], which makes Mn²⁺ ions in KMnF₃ more accessible to surrounding water molecules than that in MnO₂ (known as a classic covalent compound). The exceptional r_2 relaxivity of KMnF₃@BSA might result from the intermolecular field coupling effect caused by binding with BSA [44,45]. Generally, the intermolecular field coupling of magnetic centers (Mn²⁺) would lead to the augment of local field inhomogeneity, resulting in an increase of r_2 relaxivity [8]. Due to the covalent-ionic behavior of KMnF₃ NCs, the intermolecular field effect appears more pronounced for KMnF₃@BSA. Nonetheless, the exact mechanism of the r_2 enhancement of KMnF₃@BSA NCs requires further investigation. Apart from the relaxation

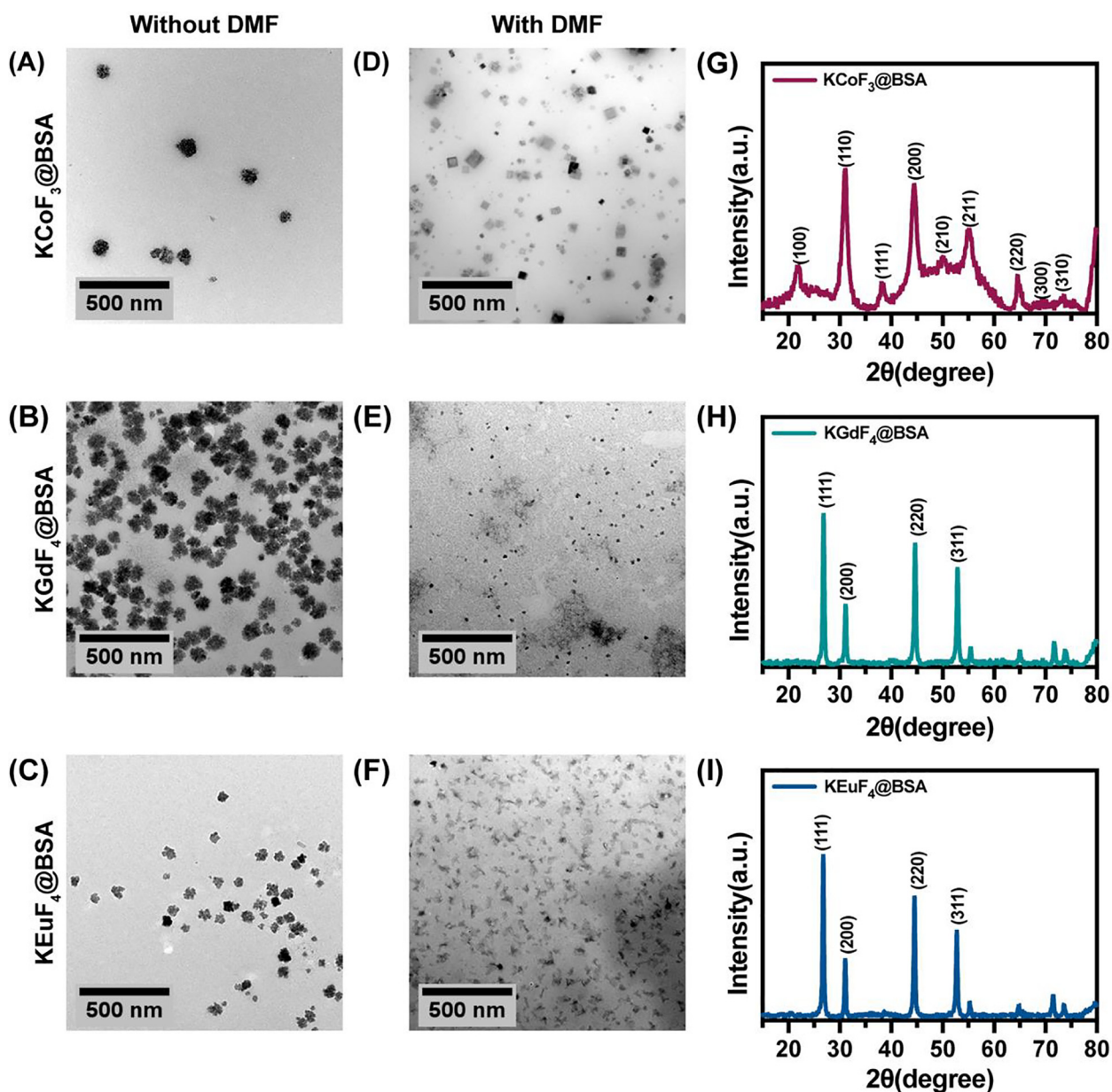


Fig. 3. TEM images of (A) and (D) KCoF₃@BSA, (B) and (E) KGdF₄@BSA, and (C) and (F) KEuF₄@BSA in the absence and presence of DMF, and (G-I) the corresponding XRD patterns of the NPs fabricated in the presence of DMF.

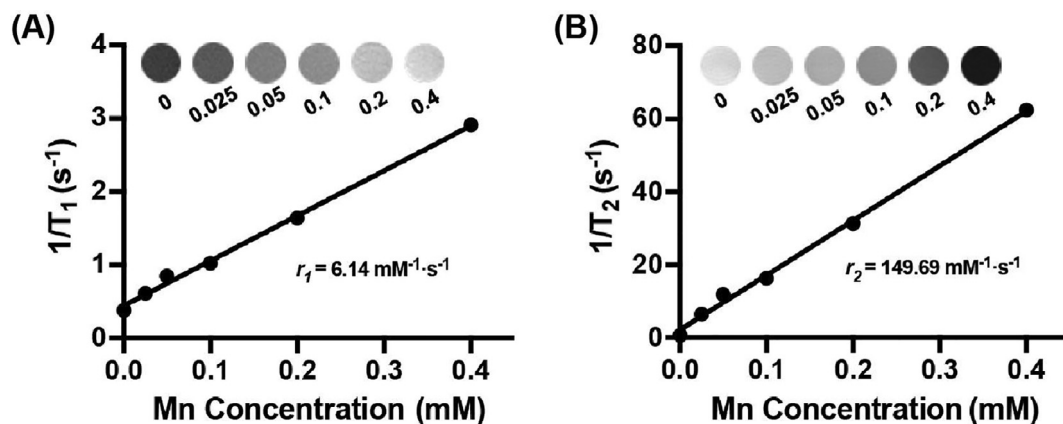


Fig. 4. Relaxation properties of KMnF₃@BSA NCs. (A) T_1 and (B) T_2 relaxation properties and corresponding phantom images.

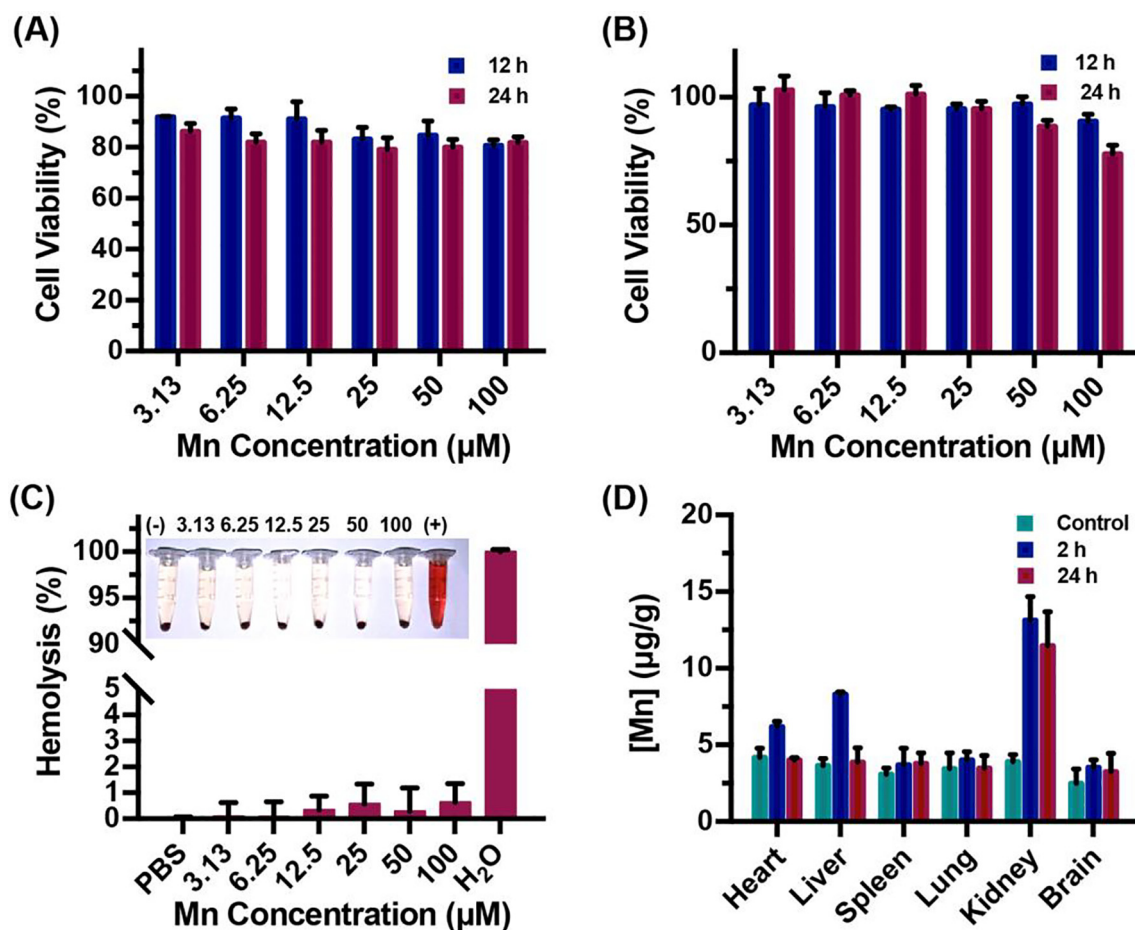


Fig. 5. *In vitro* biocompatibility and biodistribution of KMnF₃@BSA NCs. Cell viability of (A) C6 cells and (B) HUVEC upon incubation with the KMnF₃@BSA NCs at various concentrations for 12 and 24 h, (C) hemolysis analysis, and (D) biodistribution of KMnF₃@BSA NCs at 2 and 24 h post-injection.

values, the r_2/r_1 ratios are widely used for ascertaining the classification of contrast agents. It is generally accepted that when the r_2/r_1 ratio is higher than 10, the magnetic nanomaterials are referred as T_2 contrast agents. However, the high accessibility of Mn²⁺ ions to water molecules caused by the unique structure of KMnF₃@BSA NCs resulted in a strong positive contrast enhancement even with an r_2/r_1 ratio of 24.4.

3.3. *In vitro* biocompatibility

Because cytotoxicity is a major concern for nanoparticle-based contrast agents, the cell viability assay was carried out to determine the toxicity profiles of the KMnF₃@BSA NCs against human umbilical vein endothelial cells (HUVEC) and C6 glioma cells. Upon incubation with KMnF₃@BSA NCs for 12 and 24 h, the cell viability of HUVEC and C6 glioma cells remained > 80% at Mn concentrations as high as 100 μM (Fig. 5A and B). In addition, the morphologies and cell densities of HUVEC and C6 cells were observed by microscopy (Fig. S6). There was no obvious difference between the control cells and the cells treated with KMnF₃@BSA NCs in the microscopic images of both cell types. Together, these results implied that the low cytotoxicity of KMnF₃@BSA NCs made them valuable in bio-related studies. Moreover, the hemolysis assays were conducted to evaluate the toxicity of KMnF₃@BSA NCs to blood cells. The hemolysis ratio is an essential parameter to evaluate blood compatibility. Fig. 5C shows that after 2 h of incubation with erythrocytes, all KMnF₃@BSA NC dispersions at the test concentrations were non-hemolytic. The hemolysis was lower than 1% at the maximum experimental concentration, indicating the good hemocompatibility of KMnF₃@BSA NCs. Taken together, all the above results

showed the non-cytotoxic character of the formulated KMnF₃@BSA NCs, allowing them to be administered intravenously for bio-applications.

3.4. Biodistribution

To further verify the biocompatibility, the biodistribution of KMnF₃@BSA NCs was measured using a dosage of 5 mg Mn/kg in healthy ICR mice. As presented in Fig. 5D, the NCs predominantly accumulated in the kidney and liver within 2 h, with a low accumulation in the heart and brain. This implied that the KMnF₃@BSA NCs rapidly circulated in blood after intravenous injection and could be retained in organs with high levels of blood flow plus processing (e.g., liver). At 24 h after injection, KMnF₃@BSA NCs exhibited a higher distribution in the kidneys, inferring that the KMnF₃@BSA NCs could be excreted by urine. Meanwhile, the amount of Mn decreased in other organs, which basically returned to pre-injection levels. Together, these results showed that KMnF₃@BSA NCs could be eliminated from organs, and were mainly excreted via the renal clearance route, which was in a good agreement with the previously reported manganese-based NPs.

3.5. *In vivo* dual-modal MRI

The capability of KMnF₃@BSA NCs to cross the brain–blood barrier (BBB) was first evaluated on a 7.0 T MRI scanner with healthy ICR mice. As demonstrated in Fig. S7, after the intravenous administration of KMnF₃@BSA NCs, the T_1 contrast enhancement effects could be observed in the CA3 region of the hippocampus of healthy mice brains [46], indicating the permeability of the KMnF₃@BSA NCs through the

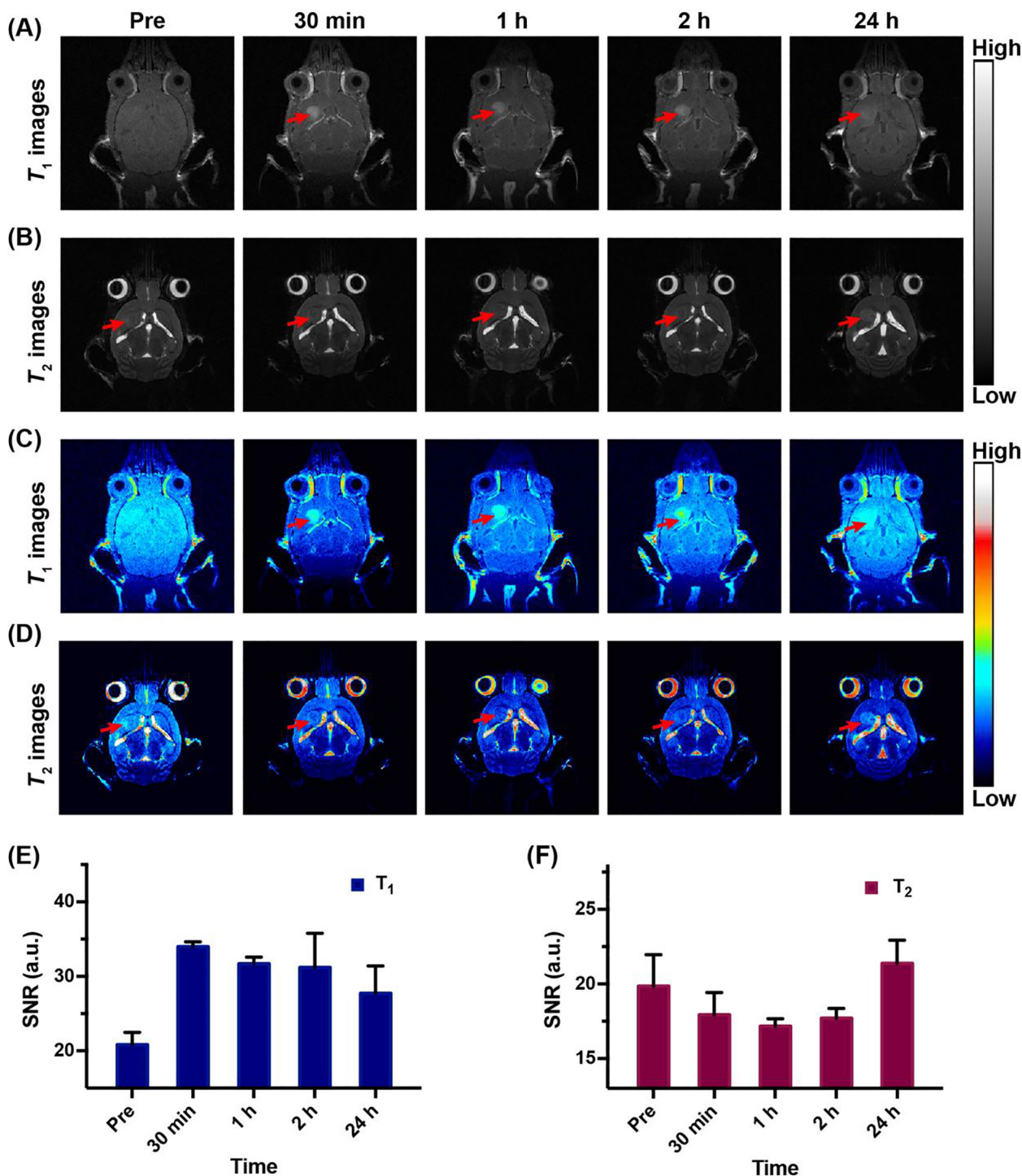


Fig. 6. T_1 - T_2 MRI of glioma bearing mouse before and after intravenous injection of $\text{KMnF}_3\text{@BSA}$ NCs at different time points (red arrows point to gliomas, dose = 5 mg Mn/kg), (A) T_1 - and (B) T_2 -weighted images, (C) and (D) pseudo-colored images, (E) and (F) SNR analyses.

BBB, which might be attributed to their conjugation of BSA. Overall, these results suggested the potential use of $\text{KMnF}_3\text{@BSA}$ NCs in brain diagnoses.

Next, T_1 - T_2 dual-modal MRI was investigated in glioma-bearing mice with a dosage of 5 mg Mn/kg ($n = 3$). At 30 min intravenous post-injection of the $\text{KMnF}_3\text{@BSA}$ NCs, a brightening contrast (Fig. 6A) was observed in T_1 -weighted and a darkening contrast (Fig. 6B) in T_2 -weighted MRI within the brain glioma. Moreover, these brightening and darkening contrast effects lasted at least 2 h, which was sufficient to obtain comprehensive diagnostic information. Consequently, not

only the contrast but also the boundary between tumor and normal tissue became much clearer in both T_1 - and T_2 -weighted MRI. Note that at 24 h, the positive contrasts of the glioma became diffuse and blurred in the T_1 -weighted images, while for the T_2 -weighted MRI, the negative contrast effects appeared as pseudo-positive ones. These phenomena were due to the sharply decrease of contrast agents in the tumor regions [6,28], which could be attributed to the metabolism of the $\text{KMnF}_3\text{@BSA}$ NCs.

We used the signal-to-noise ratio (SNR) to further quantify the signal enhancement in the glioma area by using the following formula:

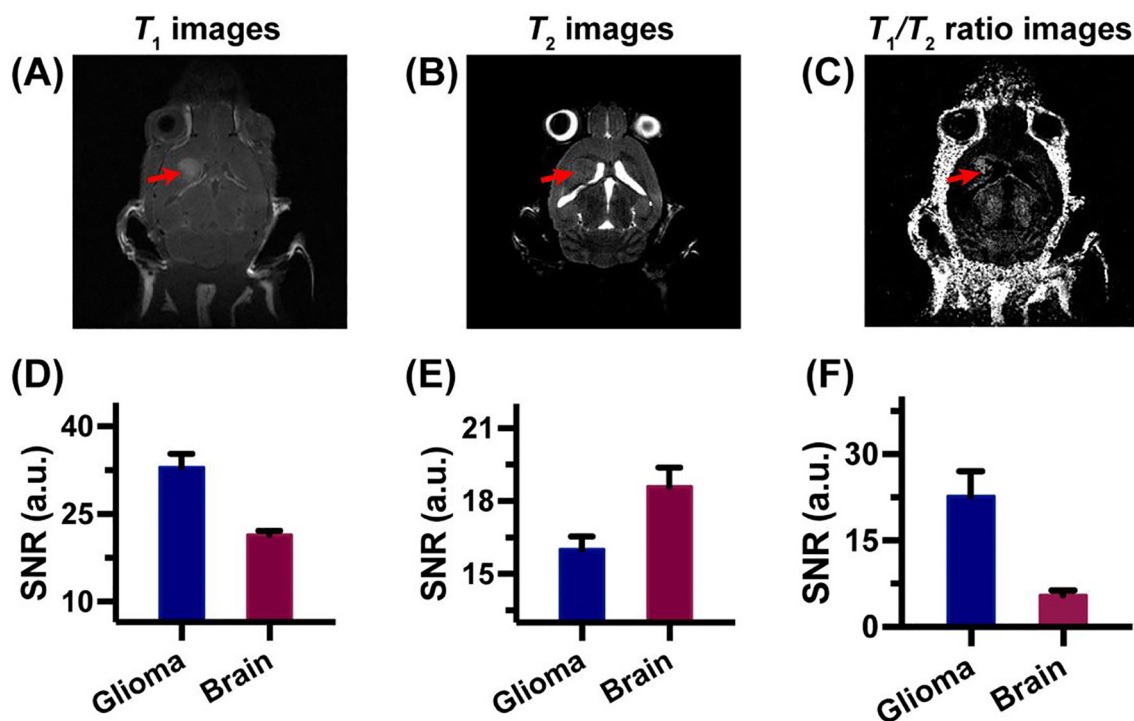


Fig. 7. Postprocessing of T_1 - and T_2 -weighted images of glioma bearing mice after the injection of $\text{KMnF}_3\text{@BSA}$ NCs to improve MRI sensitivity: (A) T_1 - and (B) T_2 -weighted images, (C) T_1/T_2 ratio images (acquired by dividing the intensity of T_1 -weighted images by the intensity of T_2 -weighted images in each corresponding pixel), (D-F) SNR analyses in glioma regions (indicated by red arrows) and surrounding brain tissue.

$\text{SNR}_{\text{glioma}} = \text{SI}_{\text{glioma}}/\text{SD}_{\text{noise}}$ (where SI means signal intensity and SD means standard deviation). As presented in Fig. 6E, for the T_1 -weighted MRI, the SNR value significantly increased to 33.98 ± 0.68 at 30 min post-injection of the $\text{KMnF}_3\text{@BSA}$ NCs, which was about 1.63-fold higher compared to the pre-injection level. Meanwhile, the SNR decreased from 19.86 ± 2.10 to 17.94 ± 1.49 at 30 min post-injection in the T_2 -weighted MRI. This thus validated that $\text{KMnF}_3\text{@BSA}$ NCs shortened the T_1 and T_2 relaxation times simultaneously and thereby could be applied as an effective dual-modal MRI contrast agent to provide complementary diagnostic information in one machine with same penetration depth and resolutions nearly simultaneously, which could hardly be achieved by the conventional clinical approved Gd^{3+} contrast agents.

Furthermore, the T_1 - T_2 dual-modal MRI is benefited from the self-confirmed ability to filter various artifacts. To show the feasibility of dual-modal $\text{KMnF}_3\text{@BSA}$ NCs for ambiguity suppression, a T_1/T_2 ratio processing of the corresponding T_1 (Fig. 7A) and T_2 (Fig. 7B) images was conducted. In the acquired T_1/T_2 ratio image (Fig. 7C), the brain glioma appeared to be much brighter while the signal of the surrounding brain tissue was greatly suppressed, validating the self-confirmed ability of T_1 - T_2 dual modal imaging. Consistently, the difference of SNR values between the glioma region and the surrounding brain tissue was significantly enhanced in the T_1/T_2 ratio image (17.27 ± 1.90), compared to those in the corresponding T_1 -weighted image (11.70 ± 0.81) and T_2 -weighted image (2.45 ± 1.32) (Fig. 7D-F). Collectively, the postprocessing of the concurrently obtained T_1 and T_2 MRI effectively eliminated the possible artifacts and greatly improved the MRI sensitivity, benefiting to accurate detection of brain gliomas with infiltrative nature.

3.6. In vivo biocompatibility

To assay the biocompatibility of the $\text{KMnF}_3\text{@BSA}$ NCs *in vivo*, the hematological analyses were first performed. After intravenous injection with $\text{KMnF}_3\text{@BSA}$ NCs, no obvious abnormal behavior and weight

loss were found in the mice for 14 days, suggesting that there was no significant acute toxicity of the injected NCs. The blood was collected for blood routine analyses and biochemistry tests at 14 days after injection. As illustrated in Fig. 8A and B, the important hepatic and renal function indices, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alanine albumin (ALB), creatinine (CRE), and blood urea nitrogen (BUN), were within the normal range with no observable differences from the control mice, verifying minimal side effects on kidney and liver. At the same time, routine blood analyses (Fig. 8B) including erythrocytes (RBC), hemameba (WBC), hemoglobin (HGB), platelets (PLT), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV) and lymphocyte (Lymph) counts also showed no distinct changes in blood parameters in the experimental group when compared with the control group, demonstrating the good hemocompatibility of $\text{KMnF}_3\text{@BSA}$ NCs.

Besides blood analyses, histopathological analyses were conducted to evaluate the potential toxicity of the $\text{KMnF}_3\text{@BSA}$ NCs to the major organs by hematoxylin and eosin (H&E) staining. As shown in Fig. 8C, no evident tissue damage, inflammation, or lesions can be seen in the major organs from mice administered with $\text{KMnF}_3\text{@BSA}$ NCs. The histological analyses further revealed that $\text{KMnF}_3\text{@BSA}$ NCs at the given dose had no/very low toxicity *in vivo* and showed good biosafety. This together with the hematological analyses results provided a preliminary validation the biocompatibility of $\text{KMnF}_3\text{@BSA}$ NCs for *in vivo* biomedical applications.

4. Conclusion

Albumin-mediated $\text{KMnF}_3\text{@BSA}$ NCs, which showed good biocompatibility and colloidal stability, were successfully synthesized through a simple “one-pot” biomimetic method. We found that the introduction of DMF in the reaction process strongly influenced the size and morphology of formed $\text{KMnF}_3\text{@BSA}$ NCs. The discovery opens new

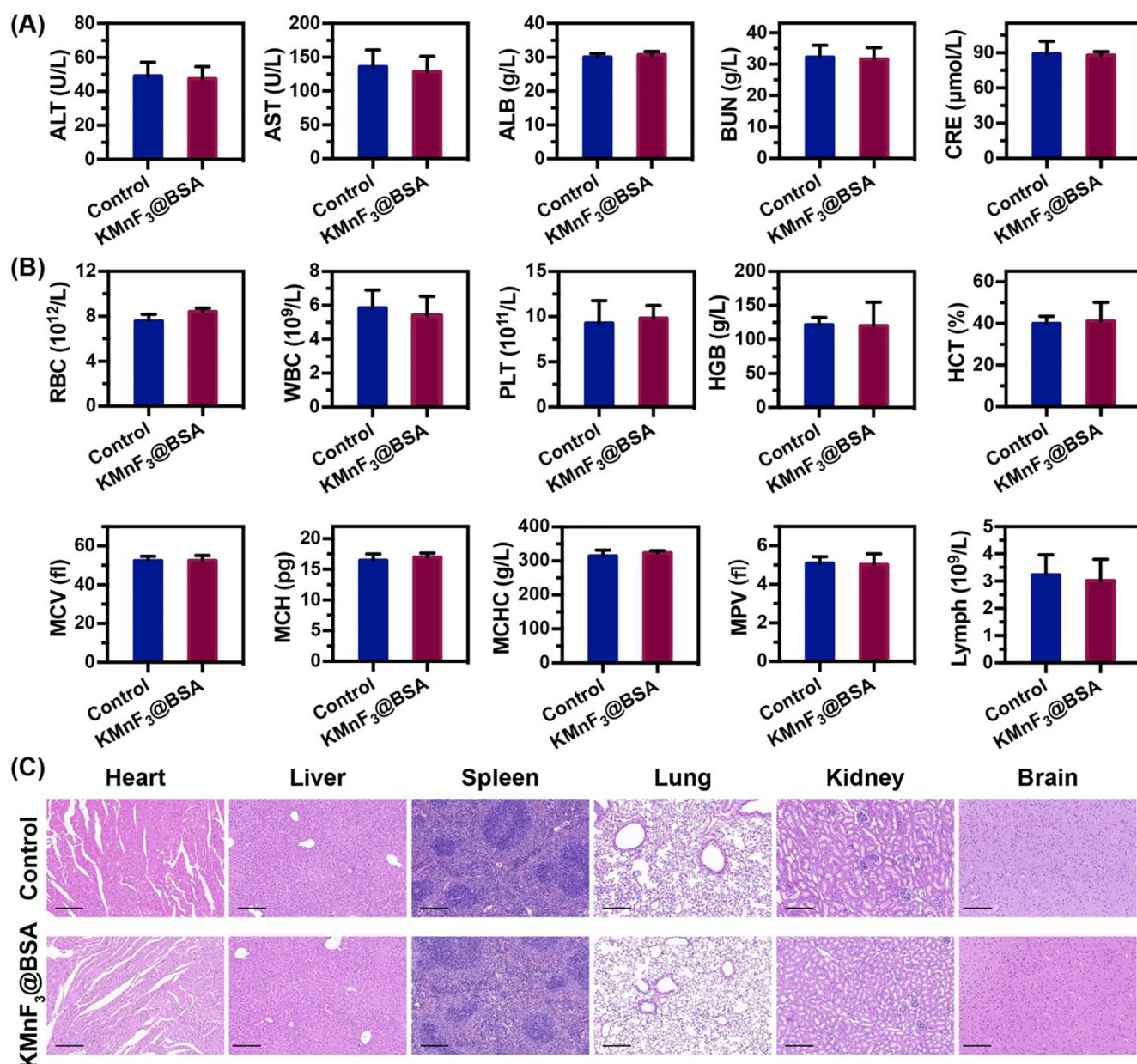


Fig. 8. *In vivo* biocompatibility. (A) blood biochemistry test, (B) routine blood analysis, and (C) H&E stained images of tissues (heart, liver, spleen, lung, kidney, and brain) of the mice harvested from the control and 14 days after intravenous injection of KMnF₃@BSA NCs. Scale bars: 200 μm.

opportunities for the synthesis of highly water-dispersible, size-tuned complex metal fluorides. The as-prepared KMnF₃@BSA NCs exhibited an r_1 value of 6.14 mM⁻¹s⁻¹ and an r_2 value of 149.69 mM⁻¹s⁻¹ and led to brightening contrast in T_1 images and darkening contrast in T_2 images in a brain glioma-bearing mouse model. Further correlation calculations based on the concurrently obtained T_1 and T_2 images by taking T_1/T_2 ratio greatly improved the MRI sensitivity by eliminating the possible artifacts. The dual-modal KMnF₃@BSA NCs with enhanced MRI sensitivity therefore have great promise in accurate and early detection of brain gliomas.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors gratefully acknowledge the financial support from the National Natural Science Foundation of China (81271639) and Beijing Natural Science Foundation (7162023). The supports from the Core Facility Center (CFC) at Capital Medical University are greatly acknowledged. We are also grateful for the support from Beijing Area Major Laboratory of Peptide and Small Molecular Drugs.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cej.2020.125066>.

References

- [1] X.J. Han, K. Xu, O. Taratula, K. Farsad, Applications of nanoparticles in biomedical imaging, *Nanoscale* 11 (3) (2019) 799–819.

- [2] F. Yang, P.G. Lei, J. Jiao, Recent advances in the use of magnetic nanoparticles in bio-imaging applications, *Nanosci. Nanotechnol. Lett.* 11 (7) (2019) 901–922.
- [3] D.L. Ni, W.B. Bu, E.B. Ehlerding, W.B. Cai, J.L. Shi, Engineering of inorganic nanoparticles as magnetic resonance imaging contrast agents, *Chem. Soc. Rev.* 46 (23) (2017) 7438–7468.
- [4] J. Wahsner, E.M. Gale, A. Rodriguez-Rodriguez, P. Caravan, Chemistry of MRI contrast agents: current challenges and new frontiers, *Chem. Rev.* 119 (2) (2019) 957–1057.
- [5] F. Hu, Y.S. Zhao, Inorganic nanoparticle-based T_1 and T_1/T_2 magnetic resonance contrast probes, *Nanoscale* 4 (20) (2012) 6235–6243.
- [6] Z. Zhou, D. Huang, J. Bao, Q. Chen, G. Liu, Z. Chen, X. Chen, J. Gao, A synergistically enhanced T_1 – T_2 dual-modal contrast agent, *Adv. Mater.* 24 (46) (2012) 6223–6228.
- [7] T.H. Shin, J.S. Choi, S. Yun, I.S. Kim, H.T. Song, Y. Kim, K.I. Park, J. Cheon, T_1 and T_2 dual-mode MRI contrast agent for enhancing accuracy by engineered nanomaterials, *ACS Nano* 8 (4) (2014) 3393–3401.
- [8] Z. Zhou, R. Bai, Z. Wang, H. Bryant, L. Lang, H. Merkle, J. Munasinghe, L. Tang, W. Tang, R. Tian, G. Yu, Y. Ma, G. Niu, J. Gao, X. Chen, An albumin-binding T_1 – T_2 dual-modal MRI contrast agents for improved sensitivity and accuracy in tumor imaging, *Bioconjugate Chem.* 30 (6) (2019) 1821–1829.
- [9] N. Xiao, W. Gu, H. Wang, Y. Deng, X. Shi, L. Ye, T_1 – T_2 dual-modal MRI of brain gliomas using PEGylated Gd-doped iron oxide nanoparticles, *J. Colloid Interface Sci.* 417 (2014) 159–165.
- [10] G. Huang, H. Li, J. Chen, Z. Zhao, L. Yang, X. Chi, Z. Chen, X. Wang, J. Gao, Tunable T_1 and T_2 contrast abilities of manganese-engineered iron oxide nanoparticles through size control, *Nanoscale* 6 (17) (2014) 10404–10412.
- [11] F.F. Li, D.B. Zhi, Y.F. Luo, J.Q. Zhang, X. Nan, Y.J. Zhang, W. Zhou, B.S. Qiu, L.P. Wen, G.L. Liang, Core/shell Fe_3O_4/Gd_2O_3 nanocubes as T_1 – T_2 dual modal MRI contrast agents, *Nanoscale* 8 (25) (2016) 12826–12833.
- [12] B.C. Duan, D.D. Wang, H.H. Wu, P.P. Xu, P. Jiang, G.L. Xia, Z.B. Liu, H.B. Wang, Z. Guo, Q.W. Chen, Core-shell structured $Fe_3O_4@C@MnO_2$ nanoparticles as pH Responsive T_1 – T_2 dual-modal contrast agents for tumor diagnosis, *ACS Biomater. Sci. Eng.* 4 (8) (2018) 3047–3054.
- [13] Z. Zhou, R. Bai, J. Munasinghe, Z. Shen, L. Nie, X. Chen, T_1 – T_2 dual-modal magnetic resonance imaging: from molecular basis to contrast agents, *ACS Nano* 11 (6) (2017) 5227–5232.
- [14] S. Suarez-Garcia, N. Arias-Ramos, C. Frias, A.P. Candiota, C. Arus, J. Lorenzo, D. Ruiz-Molina, F. Novio, Dual T_1/T_2 nanoscale coordination polymers as novel contrast agents for MRI: a preclinical study for brain tumor, *ACS Appl. Mater. Interfaces* 10 (45) (2018) 38819–38832.
- [15] S. Wang, Z.J. Zhou, G.C. Yu, N. Lu, Y.J. Liu, Y.L. Dai, X. Fu, J. Wang, X.Y. Chen, Gadolinium metallofullerene-polypyrrole nanoparticles for activatable dual-modal imaging-guided photothermal therapy, *ACS Appl. Mater. Interfaces* 10 (34) (2018) 28382–28389.
- [16] H.J. Wang, D.H. Bremner, K.H. Wu, X.R. Gong, Q. Fan, X.T. Xie, H.M. Zhang, J.Z. Wu, L.M. Zhu, Platelet membrane biomimetic bufalin-loaded hollow MnO_2 nanoparticles for MRI-guided chemo-chemodynamic combined therapy of cancer, *Chem. Eng. J.* 382 (2020) 122848.
- [17] M. Zhang, T. Zheng, B.L. Sheng, F. Wu, Q.C. Zhang, W.T. Wang, J. Shen, N.L. Zhou, Y. Sun, Mn^{2+} complex-modified polydopamine- and dual emissive carbon dots based nanoparticles for in vitro and in vivo trimodality fluorescent, photothermal, and magnetic resonance imaging, *Chem. Eng. J.* 373 (2019) 1054–1063.
- [18] D.P.J. Pan, S.D. Caruthers, A. Senpan, A.H. Schmieder, S.A. Wickline, G.M. Lanza, Revisiting an old friend: manganese-based MRI contrast agents, *Wiley Interdiscip. Rev.-Nanomed. Nanobiotechnol.* 3 (2) (2011) 162–173.
- [19] D. Niu, X. Luo, Y. Li, X. Liu, X. Wang, J. Shi, Manganese-loaded dual-mesoporous silica spheres for efficient T_1 - and T_2 -weighted dual mode magnetic resonance imaging, *ACS Appl. Mater. Interfaces* 5 (20) (2013) 9942–9948.
- [20] J.M. Shin, R.M. Anisur, M.K. Ko, G.H. Im, J.H. Lee, I.S. Lee, Hollow manganese oxide nanoparticles as multifunctional agents for magnetic resonance imaging and drug delivery, *Angew. Chem. Int. Ed. Engl.* 48 (2) (2009) 321–324.
- [21] Z.J. Liu, X.X. Song, Q. Tang, Development of PEGylated $KMnF_3$ nanoparticles as a T_1 -weighted contrast agent: chemical synthesis, in vivo brain MR imaging, and accounting for high relaxivity, *Nanoscale* 5 (11) (2013) 5073–5079.
- [22] J. Wang, F. Wang, C. Wang, Z. Liu, X. Liu, Single-band upconversion emission in lanthanide-doped $KMnF_3$ nanocrystals, *Angew. Chem. Int. Ed. Engl.* 50 (44) (2011) 10369–10372.
- [23] Y.L. Zhang, F. Wang, Y.B. Lang, J. Yin, M.L. Zhang, X.H. Liu, D.M. Zhang, D. Zhao, G.S. Qin, W.P. Qin, $KMnF_3:Yb^{3+}, Er^{3+}@KMnF_3:Yb^{3+}$ active-core-active-shell nanoparticles with enhanced red upconversion fluorescence for polymer-based waveguide amplifiers operating at 650 nm, *J. Mater. Chem. C* 3 (38) (2015) 9827–9832.
- [24] L.N. Hao, K. Liu, S. Cheng, Y. Wang, Y.J. Xu, H.S. Qian, $KMnF_3$ nanowires and nanoparticles: Selected synthesis, characterization and magnetic properties, *Mater. Lett.* 196 (2017) 145–148.
- [25] J. Sheng, K.B. Tang, D. Su, S.Y. Zeng, Y.X. Qi, H.G. Zheng, Shape-selected synthesis, characterization and optical properties of $KMnF_3$ micropolyhedra, microspheres and hollow microspheres, *J. Fluorine Chem.* 130 (8) (2009) 742–748.
- [26] L. Lei, J.J. Zhou, J.J. Zhang, S.Q. Xu, The use of zinc ions to control the size of $Yb/Er:KMnF_3$ nanocrystals with single band emission, *CrystEngComm* 17 (44) (2015) 8457–8462.
- [27] H. Colfen, Biomaterialization: a crystal-clear view, *Nat. Mater.* 9 (12) (2010) 960–961.
- [28] W. Yang, W. Guo, W. Le, G. Lv, F. Zhang, L. Shi, X. Wang, J. Wang, S. Wang, J. Chang, B. Zhang, Albumin-bioinspired Gd:CuS nanotheranostic agent for in vivo photoacoustic/magnetic resonance imaging-guided tumor-targeted photothermal therapy, *ACS Nano* 10 (11) (2016) 10245–10257.
- [29] C. Shao, S. Li, W. Gu, N.Q. Gong, J. Zhang, N. Chen, X.Y. Shi, L. Ye, Multifunctional gadolinium-doped manganese carbonate nanoparticles for targeted MR/fluorescence imaging of tiny brain gliomas, *Anal. Chem.* 87 (12) (2015) 6251–6257.
- [30] K. Liu, X. Shi, T.J. Wang, P.H. Ai, W. Gu, L. Ye, Terbium-doped manganese carbonate nanoparticles with intrinsic photoluminescence and magnetic resonance imaging capacity, *J. Colloid Interface Sci.* 485 (2017) 25–31.
- [31] J. Iwatani, T. Ishida, T. Donishi, S. Ukai, K. Shinosaki, M. Terada, Y. Kaneoke, Use of T_1 -weighted/ T_2 -weighted magnetic resonance ratio images to elucidate changes in the schizophrenic brain, *Brain Behav.* 5(10) (2015).
- [32] S.H. Xue, H. Yang, J.J. Qiao, F. Pu, J. Jiang, K. Hubbard, K. Hekmatyar, J. Langley, M. Salarian, R.C. Long, R.G. Bryant, X.P. Hu, H.E. Grossniklaus, Z.R. Liu, J.J. Yang, Protein MRI contrast agent with unprecedented metal selectivity and sensitivity for liver cancer imaging, *Proc. Natl. Acad. Sci. U. S. A.* 112 (21) (2015) 6607–6612.
- [33] Q. Jin, J. Liu, W. Zhu, Z. Dong, Z. Liu, L. Cheng, Albumin-assisted synthesis of ultrasmall Fe_2 nanodots for imaging-guided photothermal enhanced photodynamic therapy, *ACS Appl. Mater. Interfaces* 10 (1) (2018) 332–340.
- [34] A. Albanese, P.S. Tang, W.C.W. Chan, The effect of nanoparticle size, shape, and surface chemistry on biological systems, *Annu. Rev. Biomed. Eng.* 14 (1) (2012) 1–16.
- [35] F.M. Kievit, M.Q. Zhang, Cancer nanotheranostics: improving imaging and therapy by targeted delivery across biological barriers, *Adv. Mater.* 23 (36) (2011) H217–H247.
- [36] T. Kang, F.Y. Li, S. Baik, W. Shao, D.S. Ling, T. Hyeon, Surface design of magnetic nanoparticles for stimuli-responsive cancer imaging and therapy, *Biomaterials* 136 (2017) 98–114.
- [37] Q. Tang, J.M. Shen, W.J. Zhou, J.W. Liu, Z.P. Liu, Y.T. Qian, Fabrication, structure and magnetic properties of fluoroperovskite $KMnF_3$ nanostructures, *Inorg. Chem. Commun.* 7 (2) (2004) 283–285.
- [38] L.K. Bharat, G. Nagaraju, G.S.R. Raju, Y.K. Han, J.S. Yu, Shape-tunable selective synthesis of bismuth fluoride nanostructures for versatile applications, *Part. Part. Syst. Charact.* 35 (6) (2018) 9.
- [39] M. Miyake, K. Miyabayashi, Shape and size controlled Pt nanocrystals as novel model catalysts, *Catal. Surv. Asia* 16 (1) (2012) 1–13.
- [40] D. Konopacka-Lyskawa, B. Koscielska, J. Karczewski, Controlling the size and morphology of precipitated calcite particles by the selection of solvent composition, *J. Cryst. Growth* 478 (2017) 102–110.
- [41] C. Cao, S.L. Guo, B.K. Moon, B.C. Choi, J.H. Jeong, Synthesis, grouping, and optical properties of REF_3 -KF nanocrystals, *Mater. Chem. Phys.* 139 (2–3) (2013) 609–615.
- [42] P. Parhi, V. Manivannan, Novel microwave assisted solid state metathesis synthesis of KMF_3 ($M = Zn, Mn, Mg, \text{ and } Co$), *Mater. Lett.* 62 (19) (2008) 3468–3470.
- [43] Hayatullah, G. Murtaza, R. Khenata, S. Muhammad, A.H. Reshak, K.M. Wong, S. Bin Omran, Z.A. Alahmed, Structural, chemical bonding, electronic and magnetic properties of KMF_3 ($M = Mn, Fe, Co, Ni$) compounds, *Comput. Mater. Sci.* 85 (2014) 402–408.
- [44] L.R. Wang, H.Y. Lin, L.C. Ma, J.B. Jin, T.P. Shen, R.X. Wei, X.M. Wang, H. Ai, Z. Chen, J.H. Gao, Albumin-based nanoparticles loaded with hydrophobic gadolinium chelates as T_1 – T_2 dual-mode contrast agents for accurate liver tumor imaging, *Nanoscale* 9 (13) (2017) 4516–4523.
- [45] Z.J. Hai, Y.H. Ni, D. Saimi, H.Y. Yang, H.Y. Tong, K. Zhong, G.L. Liang, gamma-Glutamyltranspeptidase-triggered intracellular gadolinium nanoparticle formation enhances the T_2 -weighted MR contrast of tumor, *Nano Lett.* 19 (4) (2019) 2428–2433.
- [46] A. Kamnakh, M.D. Budde, E. Kovessdi, J.B. Long, J.A. Frank, D.V. Agoston, Diffusion tensor imaging reveals acute subcortical changes after mild blast-induced traumatic brain injury, *Sci. Rep.* 4 (2015) 4809.