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<https://doi.org/10.1038/s42004-019-0199-z>

OPEN

Synthesis of calcium carbonate nanoparticles in erythrocytes enables efficient removal of extracellular lead ions

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The “cyborg cells”, living cells with built-in nanoscaffolds, which could integrate the biological function of the cells with the functionality of nanomaterials, have been rarely explored. Here we report a method to construct “cyborg erythrocytes” through the in situ reaction of exogenous calcium and carbonate ions to generate calcium carbonate nanodots inside erythrocytes. The intracellular calcium carbonate nanodots combined with proteins are hidden under the membrane of erythrocytes, which can restrict migration and unexpected accumulation of nanodots in the body, improving the biosecurity of the nanodots. Most importantly, the calcium carbonate nanodots not only do not bring adverse effects on the nature of erythrocytes, but also endow erythrocytes with new properties. The in vitro and in vivo results show that the “cyborg erythrocytes” can remove 80% of lead ions in a blood poisoning model and reduce the lead level in the kidney and liver of mice through a precipitation transformation mechanism.

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Inorganic nanominerals produced by biomineralization under the mediation of living cells^{1–3} have important biological functions, for example, biomineralized intracellular Fe₃O₄/Fe₃S₄ nanocrystals render the magnetotactic bacteria the capability to sense the geomagnetic field^{4,5}. Inspired by the biomineralization process, the functionalization of living cells with polymers coatings or hard nanomaterial shells have received considerable attention^{6–8}. Such “cyborg cells”, nanomaterial-functionalized cells, combine the biological function of the cells with the functionality of the nanomaterials affording novel functions^{9–11}. For example, the vaccines with calcium phosphate exterior become thermostable at room temperature¹². The yeast cells with MnO₂ coating could effectively protect the encased cells from long-term physical and chemical stressor¹³. However, the functionalized cells, living cells with built-in nanomaterials, have been rarely explored. Current researches are mainly concentrated on utilizing different microbes, such as bacteria^{14,15}, fungi^{16,17} and viruses^{18,19} as reaction sites to prepare inorganic nanomaterials rather than for the construction of the nanomaterial-functionalized cells^{20,21}. Especially, because of the complexity and fragility, the use of human cells remains a great challenge^{22,23}.

Our attention on erythrocytes arises from the fact that they are important cells for oxygen transportation in human bodies^{24,25} and featured by long circulating half-life, membrane selective permeability, flexibility and stability, big cell cavities and high surface-to-volume ratio^{26,27}. Thus we consider to build “cyborg erythrocytes”, living cells with built-in stealth CaCO₃ nanodots (NDs). Such intracellular NDs can well combine with proteins and are stealth under the cover of erythrocytes, which can restrict the migration of NDs and avoid the unexpected accumulation of NDs in the body, improving their biosafety. The “cyborg erythrocytes” maybe applied to biomedical fields, such as removal of heavy metals lead, which are difficult to be removed once they enter into the body, but can cause various diseases^{28,29}. Presently, the clinical method to remove heavy metals from the body generally involves organic chelators such as ethylenediaminetetraacetic acid (EDTA) and 2,3-dimercaptosuccinic acid (DMSA)^{30,31}. However, the chelators, which are lack of selectivity, will simultaneously extract essential metals (i.e. iron, magnesium, calcium and zinc) from the body, resulting in mineral deficiencies^{32,33}.

In this study, we successfully construct functionalized erythrocytes through the synthesis of stealth CaCO₃ nanoscaffold with a diameter of about 3.90 nm inside the erythrocytes, and examine their capability to remove Pb²⁺ ions in vitro and in vivo. Our results show that the resulting nanodots-functionalized erythrocytes work well in reducing the accumulation of heavy metals in kidney and liver of mice through a precipitation transformation mechanism, and most importantly such ultra-small NDs could be excreted via the renal urinary route³⁴.

Results

Distribution of CaCO₃ NDs in “cyborg erythrocytes”. CaCO₃ nanoscaffolds are synthesized through two-step sequential permeation of Ca²⁺ and CO₃^{2–} into erythrocytes. The powder X-ray diffraction (XRD) (Supplementary Fig. 1) of the intracellular NDs shows that all the diffraction peaks can be indexed to calcite (JCPDS 05-0586). Transmission electron microscopy (TEM) observation shows that the intracellular CaCO₃ are well-dispersed NDs with the uniform size of 3.89 ± 0.03 nm (Fig. 1a), which is difficult for other traditional synthesis methods. The high-resolution TEM (HRTEM) images of several intracellular CaCO₃ NDs also show that the lattice fringe spacing is 0.210 nm, corresponding to the (202) plane of the calcite (Fig. 1b). Moreover, the selected area electron diffraction (SAED) pattern of the intracellular CaCO₃ NDs (the inset in Fig. 1b) exhibits the

obvious diffraction spots, further confirming the formation of the crystalline CaCO₃. Field emission scanning electron microscopy (FESEM) analysis also shows that the functionalized erythrocytes surface is as smooth as native erythrocytes surface, indicating that there is no CaCO₃ synthesized outside the erythrocytes (Fig. 1c and Supplementary Fig. 2). Furthermore, X-ray energy dispersive spectroscopy (EDS) analyses show that the surface elements of the functionalized erythrocytes remain unchanged after the intracellular synthesis (Supplementary Fig. 3). Confocal laser scanning microscopy (CLSM) was used to identify the presence of CaCO₃ NDs inside erythrocytes by staining the functionalized erythrocytes with tetracycline hydrochloride, which can coordinate with Ca²⁺ to emit yellow-green fluorescence when excited at 405 nm. From confocal images at different Z-axis focal planes (Fig. 1d–i), the intensity of yellow-green fluorescence is firstly strengthened and then weakened with the increase of Z-axis, suggesting that the CaCO₃ NDs are located inside the erythrocytes. The 3D visualization and fluorescence intensity images of functionalized erythrocytes further confirmed the location of biogenic CaCO₃ NDs inside erythrocytes (Fig. 1j, k). In contrast, the control native erythrocytes cannot emit the obvious fluorescence (Supplementary Fig. 4), indicating the absence of CaCO₃ NDs. From these results, it can be concluded that the CaCO₃ NDs are successfully synthesized inside the erythrocytes. Thermogravimetric (TG) curves of the functionalized erythrocytes and the control erythrocytes indicate that the amount of CaCO₃ NDs inside the functionalized erythrocytes is about 11 wt% (Supplementary Fig. 5).

Because Ca²⁺ and CO₃^{2–} react in the presence of endogenous hemoglobin (Hb), to explore the possible interaction of Hb with Ca²⁺ ions and intracellular CaCO₃, we recorded the Fourier transform infrared (FT-IR) spectra of erythrocytes adsorbed Ca²⁺ and intracellular CaCO₃ NDs separated from functionalized erythrocytes, respectively (Supplementary Fig. 6). As shown in the FT-IR spectra, the intracellular CaCO₃ NDs display peaks at 712 cm^{–1} and 872 cm^{–1} which originate from the ν_4 (the in-plane bending) and ν_2 (the out-of-plane bending) modes of CO₃^{2–} in calcite^{35,36}. And compared with the FT-IR spectrum of pure Hb, the absorption peaks of amide I band (1643 cm^{–1}, mainly C=O stretching) and amide II band (1527 cm^{–1}, C–N stretching coupled with N–H bending modes) both shift to low wavenumber³⁷. The spectra for Ca²⁺-Hb shows that the peak positions of amide I shift from 1656 to 1638 cm^{–1} and amide II shift from 1533 to 1530 cm^{–1} with the addition of Ca²⁺ ions. These results could indicate that there are interactions between Ca²⁺ and Hb, CaCO₃ NDs and Hb after the permeation of Ca²⁺ and CO₃^{2–} into erythrocytes in turn. The interaction between CaCO₃ NDs and Hb might restrict the migration and accumulation of NDs in the body, and improve their biosafety.

The properties of erythrocytes in response to the introduction of CaCO₃ NDs are unchanged.

The cell membrane and oxygen transport properties of functionalized erythrocytes proved immune from any detectable change. From the optical images of native and functionalized erythrocytes (Supplementary Fig. 7), it can be found that the morphology of functionalized erythrocytes remain largely intact after the intracellular synthesis of CaCO₃ NDs, and no aggregation is observed. In addition, both show good dispersity although the color of functionalized erythrocytes is slightly deeper due to the existence of intracellular CaCO₃ NDs. Furthermore, the integrity of the cell membrane was evaluated by using the osmotic fragility test. From Fig. 2a, the osmotic fragility curve (OFC) of functionalized erythrocytes is almost the same with that of the native erythrocytes, indicating the similar rupture profiles and the intact membrane properties of functionalized

Fig. 1 Characterization of “cyborg erythrocytes” and CaCO_3 NDs. **a** TEM image of intracellular CaCO_3 NDs; inset: the corresponding size distribution histogram of CaCO_3 NDs. **b** HRTEM image of several individual CaCO_3 NDs (inset: SAED pattern of CaCO_3 NDs, bottom: magnified image of the area denoted by the red dashed boxes). **c** FESEM image of functionalized erythrocytes. **d–i** Confocal laser scanning images of functionalized erythrocytes stained with tetracycline hydrochloride (excited at 405 nm, emitted at 530 nm). **d** The bright-field. **e–i** The overlay images of functionalized erythrocytes with different z-axis focal planes. **j** 3D visualization of functionalized erythrocytes. **k** Fluorescence intensity images of functionalized erythrocytes. **a** Scale bars 50 nm. **b** Scale bars 5 nm (top) and 1 nm (bottom). **c** Scale bars 5 μm . **d–i** Scale bars 5 μm . ND nanodot, TEM transmission electron microscopy, HRTEM high-resolution transmission electron microscopy

erythrocytes. Moreover, the proteins on erythrocytes plasma membrane also remained unchanged after the formation of intracellular CaCO_3 NDs. We incubated functionalized erythrocytes in the autologous serum. As shown in Fig. 2b, similar to native erythrocytes (black line), functionalized erythrocytes do not show obvious hemolysis in fresh autologous serum (red line). Compared to complete hemolysis (green line), the hemolysis ratio of functionalized erythrocytes and native erythrocytes are 4.9% and 3.4%, respectively, indicating no adverse effect on the complement-controlling proteins of the membranes during the

construction process of functionalized erythrocytes³⁸. At this stage, it can be speculated that the permeability of the erythrocyte membrane facilitate the formation of the CaCO_3 NDs. Erythrocytes are the main cells in circulation and they are responsible for transporting oxygen to body tissues. The most common cytoplasm of erythrocytes is hemoglobin, an iron-containing biomolecule that can bind oxygen. So the effects of intracellular synthesis on the hemoglobin oxygen affinity of functionalized erythrocytes were assessed. Oxygen equilibrium curves (Supplementary Fig. 8) reveal that the oxygen affinity of functionalized

Fig. 2 The properties of functionalized erythrocytes were almost unchanged compared with native erythrocytes. **a** Osmotic fragilities of native erythrocytes and functionalized erythrocytes. Each data represent the mean $\pm$ SD, $n = 3$. **b** Hemolysis of functionalized erythrocytes and control native erythrocytes by complement

erythrocytes is within the normal range (23.51 ± 1.15 mmHg) and this value is equivalent to the native erythrocytes (25.51 ± 1.17 mmHg). With the rapid development of nanotechnology, more and more attentions have been paid to the cytotoxicity and biosecurity of NDs. Herein, Chinese hamster lung cells V79 and Buffalo rat liver cells BRL 3A were selected as the model normal cells to evaluate the potential cytotoxicity of the functionalized erythrocytes, respectively. The results indicated that functionalized erythrocytes only resulted in negligible cell growth inhibition of both normal cells ($< 10\%$, Supplementary Fig. 9). Thereby, the functionalized erythrocytes can still perform their inherent responsibilities as well as the native ones and have no cytotoxicity to normal cells.

The functions of “cyborg erythrocytes”. According to American Center for Disease Control and Prevention (CDC) regulations, for children higher blood lead levels more than $100 \mu\text{g L}^{-1}$ are defined as lead poisoning. The whole blood supplemented with $200 \mu\text{g L}^{-1}$ Pb^{2+} was employed as a model sample for our experiment. As indicated by Fig. 3a, both functionalized and native erythrocytes were used to react with the poisoned blood. It can be clearly seen that nearly 80% of Pb^{2+} in blood can be immediately removed by functionalized erythrocytes during the first 10 min and the plateau is reached within 30 min. Moreover, it can be found that the adsorption capacity of functionalized erythrocytes for Pb^{2+} is much higher than that of the native ones. Equally important, while functionalized erythrocytes largely absorb Pb^{2+} , they do not reduce the concentration of the essential bulk and trace elements, such as iron, magnesium, and zinc (Fig. 3b). It can be speculated that the intracellular CaCO_3 NDs inside functionalized erythrocytes play the vital role in the removal of heavy metal ions. In addition, the functionalized erythrocytes absorbed Pb^{2+} was determined by XRD (Supplementary Fig. 10). From the XRD pattern, we can observe that part of the intracellular CaCO_3 NDs (calcite) are transformed into PbCO_3 (cerussite) after Pb^{2+} adsorption treatment. This suggests that the high adsorption capacity of functionalized erythrocytes for Pb^{2+} can be attributed to the precipitation transformation from CaCO_3 to PbCO_3 because of the great difference in the solubility product constants between calcite ($K_{\text{sp}} = 4.96 \times 10^{-9}$) and cerussite ($K_{\text{sp}} = 1.46 \times 10^{-13}$)³⁹.

In vivo animal experiments further demonstrated that functionalized erythrocytes could efficiently reduce the Pb^{2+} accumulation in the kidney and liver of mice. Two groups of 4-week-old mice were intravenously injected $100 \mu\text{L}$ of normal saline (control group, 7 per group) or saline containing 2 mg L^{-1} of Pb^{2+} (14/group). After 30 min of the injection, the blood samples were collected from each mouse for monitoring of Pb^{2+}

concentration. The mice of the second group were then regrouped (7 per group) with half intravenously injected with 30% hematocrit native erythrocytes (model group) and the other half intravenously injected $100 \mu\text{L}$ 30% hematocrit functionalized erythrocytes (test group). The control group continued to be intravenously injected with normal saline as control. Then the blood, heart, liver, spleen, lung, kidney and brain of the mice were sampled for data collection and hematoxylin-eosin (H&E) staining. Blood and part of tissue samples were digested in concentrated nitric acid until fully digested. Subsequently, the digested samples were diluted tenfold in deionized water prior to ICP-MS analysis. The Pb contents ($\mu\text{g g}^{-1}$ of wet tissue) at sacrifice (Fig. 3c) reveal the Pb accumulation in kidneys $>$ liver $>$ brain. Compared with the model group, the Pb accumulation of test groups injected with functionalized erythrocytes was greatly reduced and had no significant difference from the control group. Because the NDs smaller than 6 nm in diameter can excrete through renal urinary route⁴⁰, there was a certain amount of lead in the urine of mice (test group), while no added lead was found in the blood. This lead-induced damage was confirmed by H&E staining of the organ, which revealed that i.v. injection of native erythrocytes caused substantial damage to the liver and spleen (model group). In contrast, in the test group treated with functionalized erythrocytes, there was no obvious lesion noted in the main organs (Supplementary Fig. 11), indicating that the functionalized erythrocytes prepared in this study were endowed with a new function to reduce the Pb^{2+} accumulation in kidney and liver of mice by building the intracellular nanoscaffolds of CaCO_3 and have no obvious cytotoxicity to normal tissues in vivo.

Discussion

Based on the above results, the possible construction mechanism of functionalized erythrocytes and their function can be summarized as shown in Fig. 4. The synthesis of CaCO_3 nanoscaffolds is based on the in situ reaction of Ca^{2+} and CO_3^{2-} ions inside erythrocytes with endogenous Hb. Hb can prevent them from agglomeration and result in the successful formation of well-dispersed CaCO_3 NDs through the interaction of Hb with CaCO_3 during reaction. After 60 min of reaction at 4°C , the samples are stabilized under isotonic condition to prevent from hemolysis and then washed and collected. The low reaction temperature of 4°C is not only to preserve the function of erythrocytes but also to drive the reaction between Ca^{2+} and CO_3^{2-} ions by exploiting the permeability of erythrocyte membrane. When functionalized erythrocytes are injected into the mice model containing Pb^{2+} in vivo, Pb^{2+} ions can enter into functionalized erythrocytes just like Ca^{2+} ions and are adsorbed onto the intracellular CaCO_3

Fig. 3 The performance of “cyborg erythrocytes” to remove Pb^{2+} in vitro and in vivo. **a** Adsorption efficiency of Pb^{2+} by functionalized erythrocytes and native erythrocytes. Each data represent the mean $\pm$ SD, $n = 3$. **b** Blood contents of essential metals after 3 h of treatments as in (a), bottom is the enlarged view of the graph. **c** Pb contents in different organs of mice. The data are shown as mean $\pm$ SD. Error bars were based on at least quadruplicate measurements. p values (** $p < 0.01$) compared to no-injected Pb counterparts

NDs. Then displacement reaction occurs between Pb^{2+} and $CaCO_3$, which leads to the generation of $PbCO_3$ and the reduction of Pb^{2+} accumulation in kidney and liver of mice.

In summary, we successfully fabricated functionalized erythrocytes by building “stealth” $CaCO_3$ nanoscaffolds inside erythrocytes through a facile two-step sequential permeation of Ca^{2+} and CO_3^{2-} . The intracellular $CaCO_3$ NDs are well-dispersed ultrasmall NDs with a size of about 3.90 nm, which is difficult to form through the traditional methods. The intracellular stealth mineral nanoscaffolds could endow erythrocytes with new functions different from their native ones. Through the membrane transport of Pb^{2+} and the subsequent precipitation transformation from $CaCO_3$ to $PbCO_3$, the as-prepared functionalized erythrocytes could efficiently remove 80% of lead ions in a blood poisoning model in vitro and reduce the Pb^{2+} accumulation in kidney and liver of mice in vivo. Importantly, such ultrasmall NDs can be excreted by urine from the mice. Our research may provide a new strategy based on “cyborg cells”, for constructing a novel class of biomaterials with significant advantages.

Methods

Materials. All chemicals used in the study were of analytical grade and were used without further purification. Anhydrous calcium chloride ($CaCl_2$), sodium carbonate (Na_2CO_3) and sodium chloride (NaCl) were purchased from Chemical Reagent Company of Tianjin. Tetracycline hydrochloride (Baoman Biotechnology Co., Ltd., Shanghai, China) could coordinate with calcium ions to emit yellow-green fluorescence when excited at 405 nm, and it was used as a fluorescence probe to detect the position of $CaCO_3$ NDs. Ultrapure water was used in the whole experiment. All experiments were performed in compliance with the relevant laws and institutional guidelines, and were approved by Henan province Institutional Animal Care and Use Committee.

Synthesis of functionalized erythrocytes. Briefly, the whole blood was collected in heparin anticoagulant tubes from laboratory volunteers. Erythrocytes were separated from the whole blood by centrifugation (2000 $r \min^{-1}$, 10 min) and washed three times with cold saline before suspended in saline at a 70% hematocrit. Then 500 μL suspension of erythrocytes and 98.5 mL cold saline were added into a beaker. Then 1 mL of $CaCl_2$ aqueous solution (1.0 M, 4 $^{\circ}C$) was added and

moderately shaken for 60 min at 4 $^{\circ}C$, followed by washing twice with cold saline through centrifugation. The washed $CaCl_2$ erythrocytes were transferred to another beaker with 98.5 mL cold saline and 1 mL aqueous solution of Na_2CO_3 (1.0 M, 4 $^{\circ}C$), for reactions under the same temperature. Finally, the functionalized erythrocytes were collected and washed twice using cold saline by centrifugation. All chemicals were of analytical grade and used without further purification.

Characterization of the functionalized erythrocytes. The morphology and energy dispersive spectra (EDS) of the functionalized erythrocytes were characterized using field emission scanning electron microscopy (FESEM, Hitachi, SU8010, Japan) equipped with an EDS analyzer. XRD measurements were conducted on a Bruker D8&Advance X-ray powder diffractometer with graphite monochromatized Cu K α ($\lambda = 0.15406$ nm). A scanning rate of 0.05 $deg s^{-1}$ was applied to record the pattern in the 2θ range of 20–70 $^{\circ}$. The Fourier transform infrared (FT-IR) spectra were recorded on a FTS-40 FT-IR spectrometer in the wavenumber range of 4000–400 cm^{-1} . The spectra were collected at 2 cm^{-1} resolution with 128 scans by preparing KBr pellets with a 3:100 “sample to-KBr” ratio. The TG analysis was conducted on an EXSTARTG/DTA 6300 instrument.

Fluorescent microscopy analysis. CLSM images of erythrocytes were taken using a OLYMPUS FV1200MOE confocal laser scanning microscope (Japan). The functionalized erythrocytes were stained with tetracycline hydrochloride and observed by confocal laser scanning microscope.

Characterization of $CaCO_3$ NDs inside erythrocytes. $CaCO_3$ NDs were extracted by solubilizing the functionalized erythrocytes using a lysis buffer and the $CaCO_3$ NDs were collected from cell lysate through centrifugation. The size and morphology of the $CaCO_3$ NDs were characterized by high-resolution-TEM (HRTEM, JEOL JEM-2100) with an acceleration voltage of 200 kV. The samples were suspended in double distilled water, fully dispersed by sonication, and deposited on a piece of ultra-thin carbon-coated copper grid prior to observation.

Optical microscopy. Bright field optical images of native erythrocytes and the functionalized erythrocytes were acquired using an inverted Nikon Eclipse TE 300 microscope equipped with a Webbers MyScope M320 CCD camera. Samples were imaged immediately after preparation using standard glass slides and cover slips.

Properties of the functionalized erythrocytes. The OFC of native erythrocytes (control) and the functionalized erythrocytes were determined by adding 25 μL of each erythrocytes suspension to 2.5 mL of a series of hypotonic saline solutions with increasing osmolality 0–0.9 NaCl/% (W/V) at room temperature for about

Fig. 4 Synthesis of functionalized erythrocytes. Schematic illustration for the construction mechanism of the functionalized erythrocytes and the reduction of Pb^{2+} accumulation in kidney and liver of mice

60 min. Then the suspensions were centrifuged (800 g, 5 min) and the absorbance (A) of each supernatant was measured at 540 nm (TU-1900). The hemolysis percentage was calculated as the ratio between each A_{540} value and that of the supernatant at 0 NaCl/% (W/V). To evaluate hemolysis by complement, the functionalized erythrocytes were incubated in fresh serum for 1 h at 37 °C. The degree of hemolysis was judged by observation and spectrophotometrical determination of released hemoglobin at 540 nm.

Sorption kinetics. For sorption kinetics measurements, the whole blood was spiked with divalent heavy metal ions (Pb^{2+}) to obtain the final concentration of $200 \mu\text{g L}^{-1}$. After 30 min of incubation, it was aliquoted into 3 mL volumes in a 20 mL polypropylene vial. The mixture was then spiked with 500 μL of 30% hematocrit functionalized erythrocytes ($\approx 2.0 \times 10^9$). The sample was then shaken at 60 rpm in a thermostatic shaker. At predetermined intervals (5, 10, 30, 60, 90 min, 2, and 3 h), 0.5 mL of the supernatant was collected and digested in concentrated nitric acid until fully digested. Then the samples were diluted tenfold in deionized water prior to metal analysis. The control test was performed in the same fashion but using native erythrocytes. The metal concentrations in the control (native erythrocytes) and the test mixture (functionalized erythrocytes) were analyzed using an inductively coupled plasma-mass spectrometer (ICP-MS, ELAN DRC-e, Perkin-Elmer Sciex). All batch experiments were conducted in triplicate and the average values were used.

In vitro cytotoxicity study. Chinese hamster lung cells V79 were cultured in Dulbecco's Modified Eagle Medium (DMEM) medium containing heat-inactivated Fetal Bovine Serum (FBS) (10%), 1% penicillin/streptomycin, amphotericin B (fungizone, $0.25 \mu\text{g mL}^{-1}$) and sodium bicarbonate (2 mg mL^{-1}) at 37 °C under 5% CO_2 . Buffalo rat liver cells BRL 3A were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% FBS, 1% penicillin/streptomycin, amphotericin B (fungizone, $0.25 \mu\text{g mL}^{-1}$) and sodium bicarbonate (2 mg mL^{-1}) at 37 °C under 5% CO_2 . The in vitro cytotoxicity and biosecurity study was also evaluated using a standard methyl thiazolyl tetrazolium (MTT, Sigma Aldrich) assay for cell viability measurement. V79 or BRL 3A cells were seeded into a 96-

well cell culture plate at 1×10^4 per well until adherent and then incubated for 24 h with 50 μL of 30% hematocrit functionalized erythrocytes.

Animal experiments. The mice were placed individually in metabolic cages with temperature and humidity levels maintained constant ($21 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$, 40% humidity) during the entire period of the study. They were maintained on a 12 h light cycle (0600 to 1800 hours) with ad lib access to food and water. The lead poisoning model was determined by American Center for Disease Control and Prevention (CDC) regulations. The sample size was determined by the concentration of lead in mice and the weight of mice. Two groups of 4-week-old male and female Balb/c mice were intravenously injected 100 μL of normal saline (control group, 7 per group) or saline containing 2 mg L^{-1} of Pb^{2+} (14 per group). After 30 min, blood samples were collected from each animal to monitor Pb^{2+} concentration in the blood. Then the mice of the second group were regrouped (7 per group) with half intravenously injected 100 μL 30% hematocrit native erythrocytes (model group) and the other half intravenously injected 100 μL 30% hematocrit functionalized erythrocytes (test group). The first group continued to intravenously inject normal saline as control. Then the mice were sacrificed, and blood, heart, liver, spleen, lung, kidneys and brain were collected. Blood and part of tissue samples were digested in concentrated nitric acid until fully digested. Then the samples were diluted tenfold in deionized water prior to metal analysis by ICP-MS. The other organs were fixed in 10% formalin solution for H&E staining. After the H&E staining, the sections were examined by Eclipse TE 300 microscope (Nikon, Japan).

Data availability

The data that support the findings of this study are available within the article and its Supplementary Information files. All other relevant source data are available from the corresponding authors upon reasonable request.

Received: 14 March 2019 Accepted: 17 July 2019

Published online: 06 September 2019

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Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (21571053, 21877027, 21771058), the 111 project (D17007), Henan Center for Outstanding Overseas Scientists (GZS2018003) and the Program for Innovative Research Team in Science and Technology in University of Henan Province (19IRTSTHN023, 18IRTSTHN002). J.L. gratefully acknowledges support from the U.S. Department of Energy (DOE), Office of Energy Efficiency and Renewable Energy, Vehicle Technologies Office. Argonne National Laboratory is operated for DOE Office of Science by UChicago Argonne, LLC, under contract number DE-AC02-06CH11357.

Author contributions

L.Y., X.R. and X.X. designed and performed the experiments. F.W. and L.Z. assisted the cell culture experiment. K.W. supported the animal experiments. L.Y., Z.B. and J.L. supervised the project. X.M., Y.G. and X.R. prepared the manuscript. All authors discussed the results and commented on the manuscript.

Additional information

Supplementary information accompanies this paper at <https://doi.org/10.1038/s42004-019-0199-z>.

Competing interests: J.L. is an Editorial Board Member at Communications Chemistry, but was not involved in the editorial review of, nor the decision to publish, this article. The other authors declare no competing interests.

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