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Supplementary Information

Fabrication of Bi_2S_3 Capsules as a Highly Sensitive X-ray Contrast Agent for Gastrointestinal Motility Assessment in vivo

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Experimental Section

Chemicals and materials

All used reagents were at least analytical grade without further purification. Bi_2S_3, $BaSO_4$, Iohexol, AM251 (CB1 receptor antagonist), DMSO, PEG-400, Tween-80 were purchased from Aladdin Chemistry Corporation (Shanghai, China). Isoflurane was purchased from RWD (Shenzhen, China). Vincristine was purchased from DingGuo Biotechnology Co. Ltd. (Tianjin, China). Ultraviolet curable resin was purchased from Jiguang Erwo Technology Co. Ltd. (Shenzhen, China). Commercial gelatin capsules were purchased from Xiyao Biological Co. Ltd. (Shanghai, China). Ultrapure water was obtained from Hangzhou Wahaha Group Co. Ltd. (Hangzhou, China).

Characterization

The scanning electron microscope (SEM) images of Bi_2S_3 powder were obtained by a Merlin Compact Field-Emission-Scanning Electron Microscope (Carl Zeiss, Germany). X-ray diffraction (XRD) pattern of Bi_2S_3 powder was obtained on an Ultima Ⅳ diffractometer (Rigaku, Japan). The photos of capsules before and after filling with Bi_2S_3 powder were taken by a camera on the phone.

Synthesis of Bi_2S_3 capsules

The standard commercial gelatin capsules for mice and rats were used in this study, and the capsules were 3 mm in diameter and 8 mm in length (the cap section is about 4.5 mm and body section is about 5 mm in length) (Figure S1). Bi_2S_3 powder was filled into the gelatin capsules as

many as possible to maximize the imaging ability. To ensure the uniformity and reproducibility of the capsules, 25 mg Bi_2S_3 powder, which accounted for about 80% of the volume of capsules, was used to produce primary Bi_2S_3 capsules. Firstly, we separated the cap and the body of the capsule, and put the commercial Bi_2S_3 powder in the capsule body with a small steel spoon, followed by gently putting the cap on the body. Finally, the capsule was coated the ultraviolet-curable resin, which was solidified by the irradiation of a 405 nm ultraviolet lamp (Huiheng Technology Co. Ltd.) for 10 s to form uniform Bi_2S_3 capsules.

Synthesis of $BaSO_4$ and Iohexol capsules

Similar to the synthesis of Bi_2S_3 capsules, about 16 mg $BaSO_4$ and 18 mg iohexol powders were encapsulated into the capsules, respectively, and then the capsules were coated the ultraviolet-curable resin, which was solidified by the irradiation of a 405 nm ultraviolet lamp for 10 s to form uniform $BaSO_4$ and iohexol capsules. It should be noted that the density of $BaSO_4$ and iohexol are smaller than that of Bi_2S_3, and the capsules can carry about 16 mg $BaSO_4$, 18 mg iohexol or 25 mg Bi_2S_3 with a similar volume.

Stability assessment of Bi_2S_3 capsules

The Bi_2S_3 capsules were dispersed in dilute hydrochloric acid (pH = 1) and artificial small bowel fluid (simulated body fluid environment), and the morphology of the capsules at 37 ℃ were monitored by taken photos at different time points (0 h and 12 h).

X-ray, CT and Spectral CT Imaging in Vitro

To compared the X-ray absorption ability of Bi_2S_3, $BaSO_4$ and iohexol suspension, Bi_2S_3, $BaSO_4$ and iohexol power with the equivalent molar and mass concentrations of radiopaque elements (Bi, Ba and I) were dispersed into alginate-Ca^{2+} hydrogel (equivalent molar concentrations: 0.0125, 0.025, 0.05, 0.1 and 0.2 M Bi/Ba/I; equivalent mass concentrations: 4, 8, 16, 32 and 64 mg/mL

Bi/Ba/I). Thereafter, the X-ray imaging, CT imaging and spectral CT imaging of Bi_2S_3, $BaSO_4$ and iohexol suspensions were carried out. Then, we compared the X-ray absorption ability of Bi_2S_3, $BaSO_4$ and iohexol capsules. Next, the X-ray attenuation ability of these three kinds of capsules were evaluated in the absence and presence of food or different concentrations of iohexol solution based on X-ray imaging, CT imaging and spectral CT imaging. To make it easy to immerse the capsule into the food, 2 mL ultrapure water was added to the food to soften it. It should be noted that the combination use of contrast capsules or rods and iohexol is prefered in some cases because the gastrointestinal tract can be observed clearly.[1] Therefore, it is essential to evaluate X-ray attenuation ability of the capsules in the presence of iohexol.

The X-ray imaging was carried out on a Carestream X-ray imaging equipment (Direct View DRX4326), and the parameters were as follows: tube voltage: 58 KVp, tube current: 250 mA, exposure time: 11.2 ms.

The CT and spectral CT imaging was performed on a 2 × 192 slice dual-energy CT scanner (Somatom Force, Siemens Healthineers, Erlangen, Germany), which has two sets of orthogonally positioned X-ray tubes and data acquisition systems on one gantry. The following parameters were used for conventional CT imaging: tube voltage: 120 kV, tube current: 88 mAs, slice thickness: 3.0 mm, and field of view: 80 × 80 mm. Different tube voltages (80, 100, 120, and 140 kV) were used to assess the in vitro imaging capability of Bi_2S_3, $BaSO_4$ and iohexol suspensions and capsules. The spectral CT images were collected on the same machine using the following parameters: 250 ms rotation time; adaptive tube current; and tube voltage 90/150 Sn. All the CT and spectral CT images (0.5 mm slice thickness and 0.25 mm increment) were reconstructed using Advanced Modeled Iterative Reconstruction (ADMIRE) with an intensity of 4. Afterward, we reconstructed the virtual monochromatic image in the range of photon energy 40–150 keV with a

10 keV increment.

Cytotoxicity assessment

Mouse embryo fibroblasts (3T3-L1 cells) were incubated in DMEM cell medium with 10% fetal bovine serum (FBS), 1% streptomycin–penicillin and a humidified atmosphere of 5% CO_2 at 37 °C. Bi_2S_3 capsules were dispersed into various media (1 mL deionized water, phosphate buffered saline (PBS) solution (pH = 7.4) and diluted hydrochloric acid (pH = 1)), and the extract solutions were obtained by removing the capsules, and transferred into 3T3-L1 cells in a 96-well cell culture plate. After 12 h incubation, each well was washed with PBS and then incubated with fresh medium containing 10 μL of MTT (5 mg/mL) for 4 h. Then, the medium in each well was removed and 120 μL of DMSO was added to dissolve the purple formazan crystals. After a 10-mins mild shake, the absorbance value at 490 nm of each well was recorded through a microplate reader (Bio-Tek).

In vivo toxicity of Bi_2S_3 capsules

The histopathological analysis was employed to evaluate the in vivo toxicity of Bi_2S_3 capsules. The histopathological changes of main organs (including heart, liver, spleen, lung, kidney, stomach, intestine tract) at different time points (1,7 and 14 days) after oral three Bi_2S_3 capsules were investigated by hematoxylin-eosin (HE) staining assay.

In vivo X-ray, CT and spectral CT imaging of Gastrointestinal motility

All animal experiments were performed in compliance with the guidelines of the Animal Care and Use Committee of Tianjin Medical University, and approved by the Animal Care and Use Committee of Tianjin Medical University. Female Sprague Dawley (SD) rats were purchased from Beijing HFK Bioscience Co. Ltd (Beijing, China). According to the principle of randomization, 24 rats were divided into three groups (n = 8 in each group) including control group, vincristine

group, and vincristine & AM251 group. For the control group, rats were intraperitoneally injected with NaCl (0.9%) (0.05mg/kg) at 24 h before in vivo imaging. For vincristine group, rats were intraperitoneally injected with vincristine solution (0.05 mg/kg).[2-5] Researches have shown that paralytic ileus caused by vincristine may be prevented/treated by CB1 antagonists,[2, 6] therefore, for the vincristine & AM251 treatment group, the rats were intraperitoneally injected with cannabinoid CB1 selective antagonist AM251 (1 mg/kg) for three times, the first time: 20 min before vincristine (0.05 mg/kg) injection, the second time: 12 h after vincristine injection, the third time: 24 h after vincristine injection. In vivo imaging was carried out immediately after the final injection of AM251 (Figure S16).

The rats were anesthetized with isoflurane (3.5% induction, 1.5% maintenance). They were orally administrated with three capsules through a capsule dispenser (Jiayue Glass Instrument Sales Co. Ltd. Tianjin, China). Immediately after the capsules were administered, 2 mL of 120 mg/mL iohexol solution (X-ray Imaging) or 2 mL of 25 mg/mL iohexol solution (CT and Spectral CT imaging) was orally administered with a gavage needle to show the outline of the digestive tract and the relative position of the capsule.[1] To compare the imaging performance of Bi_2S_3 and $BaSO_4$ capsules, nine rats were divided into three groups (n = 3 in each group) including control group, vincristine group, and vincristine & AM251 group, and X-ray imaging of $BaSO_4$ capsules for gastrointestinal motility assessment was also performed. X-ray, CT and spectral CT imaging in all studies was performed at different time points (0 min, 5 min, 2 h, 4 h, 8 h, and 10 h after oral capsules).

Data analysis

One-way analysis of variance (ANOVA) with post-hoc comparisons was applied to measure the statistic comparison between three groups, Dunnett's procedure was employed to correct for

multiple comparisons. All data were analyzed by Statistical Package for the Social Sciences version 19.0 (SPSS, Chicago, IL, USA). The in vivo images were analyzed by total scores and lead scores for each group. For all analyses, $p < 0.05$ was considered as statistical significance (*$p < 0.05$, ** $p < 0.01$). (Figure 6, Figure S17).

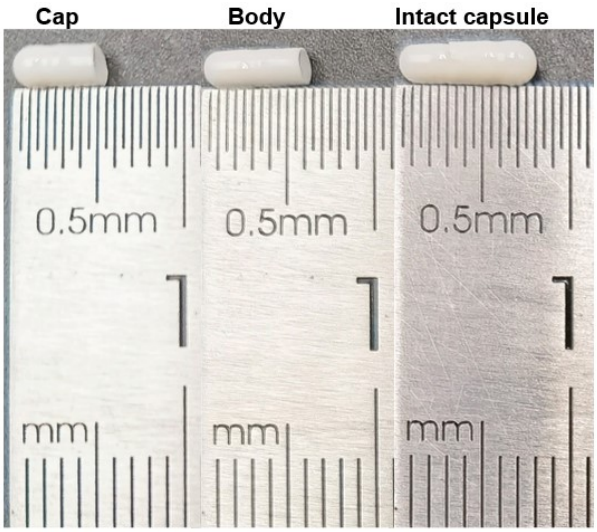

Figure S1. The photo of capsules (3 mm in diameter and 8 mm in length, cap section: 4.5 mm, body section: 5 mm).

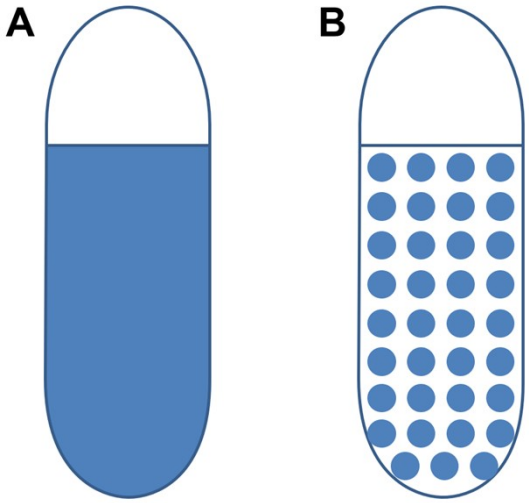

Figure S2. Schematic illustration of preparation for Bi_2S_3 crystals and powders into capsules. (A) In theory, the mass and molar concentrations of bulk Bi_2S_3 (ρ = 7.39 g/cm^3) in capsule was 6856.94 kg/m^3 and 13.33 M. (B) The mass and molar concentrations of Bi_2S_3 powder in capsule was 966.18 kg/m^3 and 1.88 M. Therefore, the concentration of pure Bi_2S_3 powder is equivalent to 14% of that of the bulk Bi_2S_3.

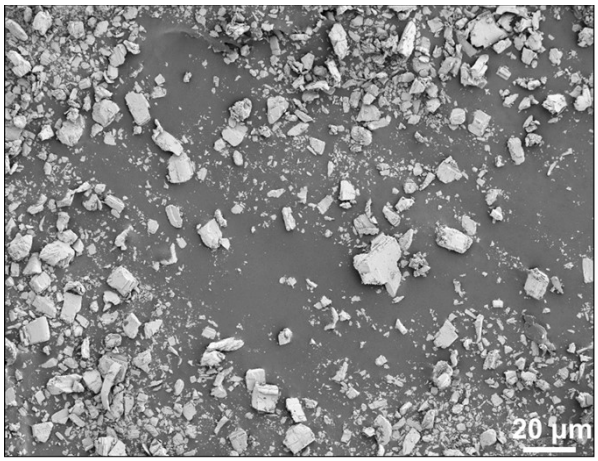

Figure S3. Scanning electron microscopy image of Bi_2S_3 powder.

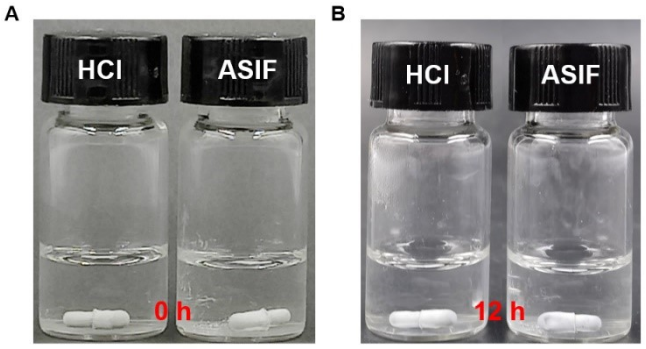

Figure S4. The stability of Bi_2S_3 capsules in stimulated gastrointestinal tract environments for 12 h. (A) Bi_2S_3 capsules immerged diluted HCl solution (pH = 1) (left), artificial small intestinal fluid (ASIF) (right) immediately. (B) Bi_2S_3 capsules can keep stable after 12 h.

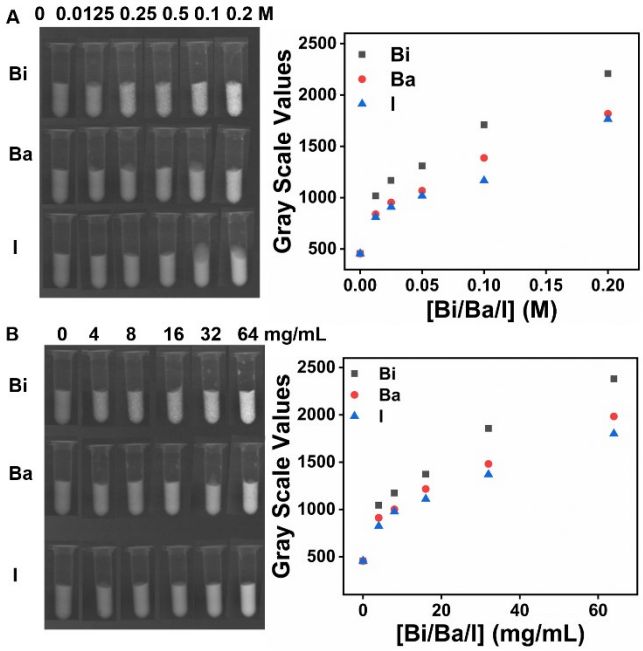

Figure S5. (A) X-ray images and gray scale values of Bi_2S_3, $BaSO_4$ and iohexol suspensions at different moalr concentrations of radioopaque elements (0.0125, 0.025, 0.05, 0.1, and 0.2 M Bi, Ba or I). (B) X-ray images and gray scale values of Bi_2S_3, $BaSO_4$ and iohexol suspensions with different mass concentrations of radioopaque elements (4, 8, 16, 32, and 64 mg/mL Bi, Ba or I).

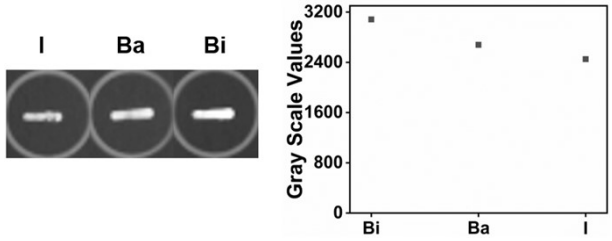

Figure S6. Gray scale values and X-ray image of Bi_2S_3, $BaSO_4$ and iohexol capsule.

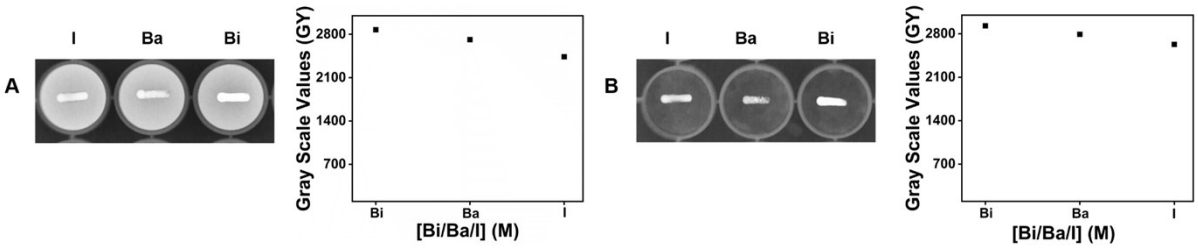

Figure S7. (A) X-ray images and gray scale values of full of Bi_2S_3, $BaSO_4$ and iohexol capsules in 120 mg/mL of iohexol solution. (B) X-ray images and gray scale values of full of Bi_2S_3, $BaSO_4$ and iohexol capsules in food.

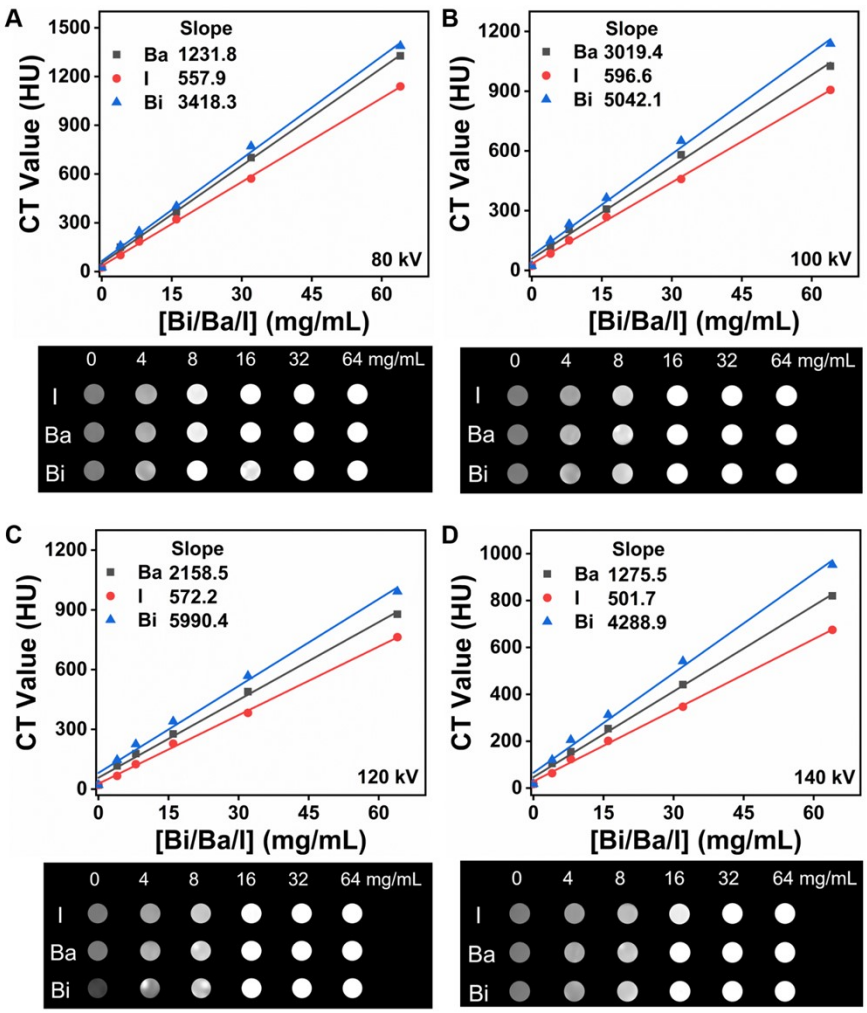

Figure S8. HU curves and CT images of Bi_2S_3, $BaSO_4$ and iohexol suspensions with different concentrations of radioopaque elements (4, 8, 16, 32, and 64 mg/mL Bi, Ba or I) at (A) 80 kV, (B) 100 kV, (C) 120 kV, (D) 140 kV.

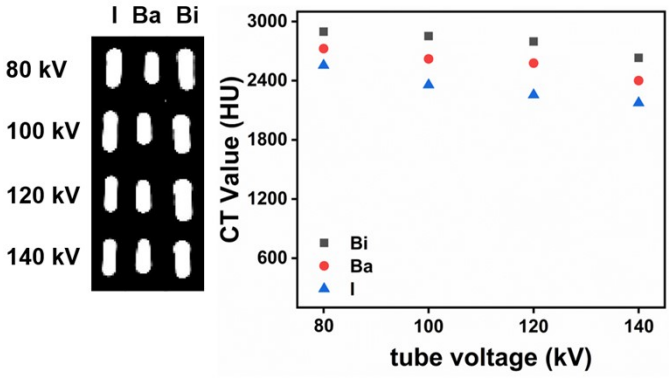

Figure S9. CT images and HU values of full of Bi_2S_3, $BaSO_4$ and iohexol capsules.

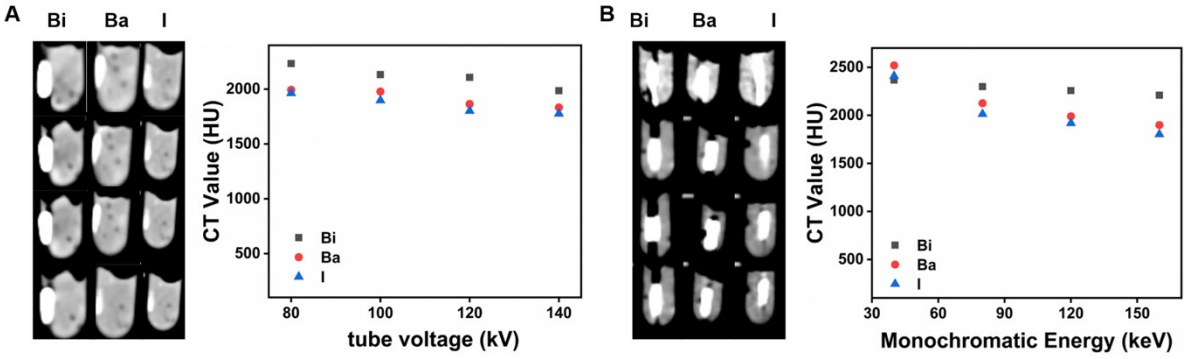

Figure S10. (A) CT images and HU values of full of Bi_2S_3, $BaSO_4$ and iohexol capsules in food. (B) Spectral CT images and HU values of full of Bi_2S_3, $BaSO_4$ and iohexol capsules in food.

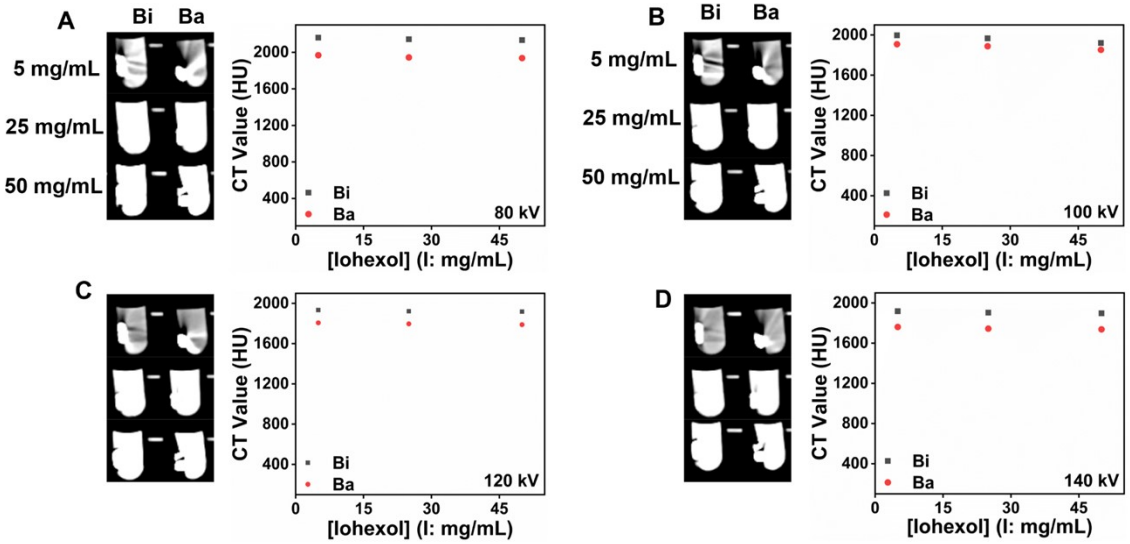

Figure S11. CT images and HU values of full of Bi_2S_3, $BaSO_4$ capsules in iohexol solution under different tube voltages.

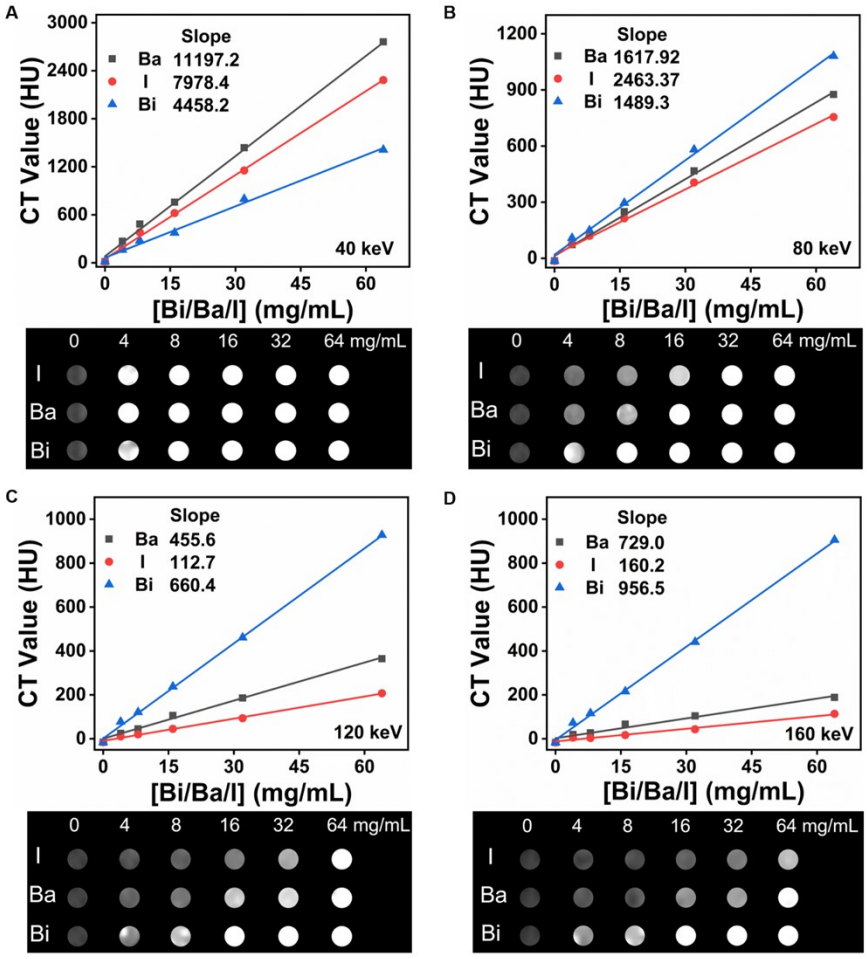

Figure S12. HU curves and spectral CT images of Bi_2S_3, $BaSO_4$ and iohexol suspensions at different concentrations of radioopaque elements (4, 8, 16, 32, and 64 mg/mL Bi, Ba or I) at (A) 40 keV, (B) 80 keV, (C) 120 keV, (D) 160 keV.

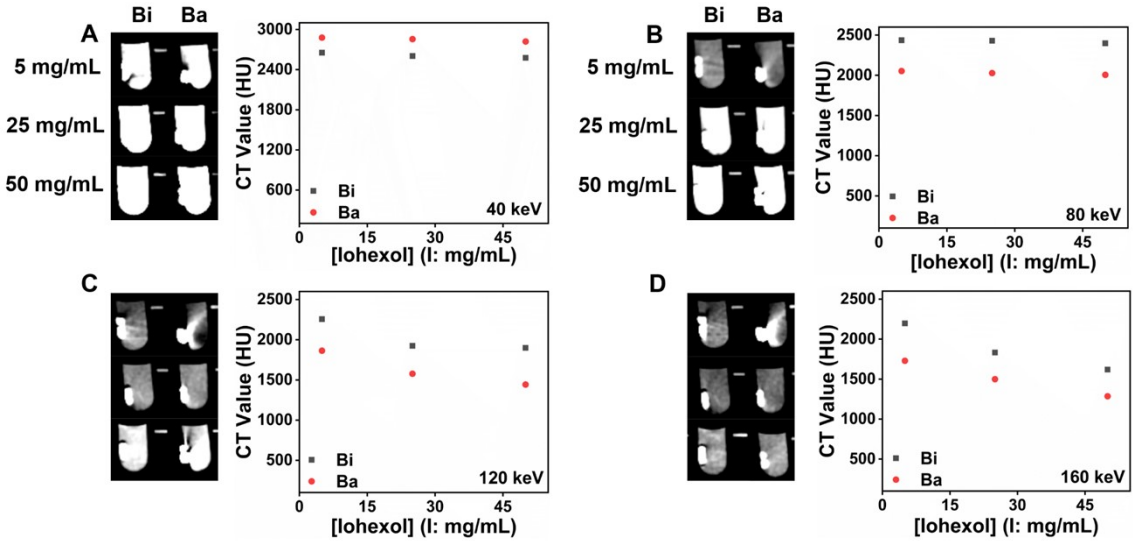

Figure S13. Spectral CT images and HU values at various X-ray energies of full of Bi_2S_3, $BaSO_4$ capsules in iohexol solution.

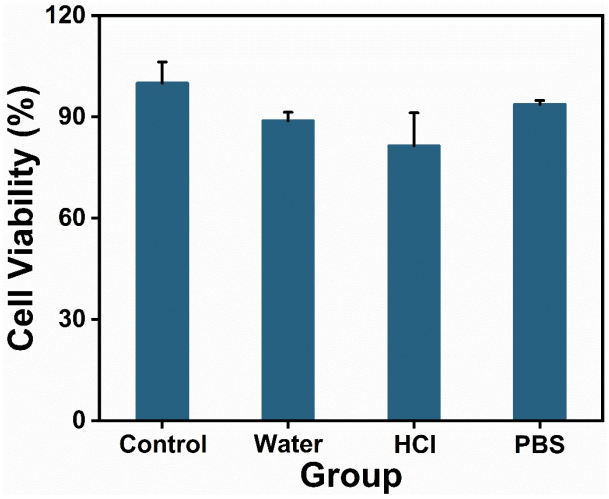

Figure S14. Cellular viabilities of 3T3-L1 cells after being treated with various leach solutions (water, HCl, PBS) for 12 h.

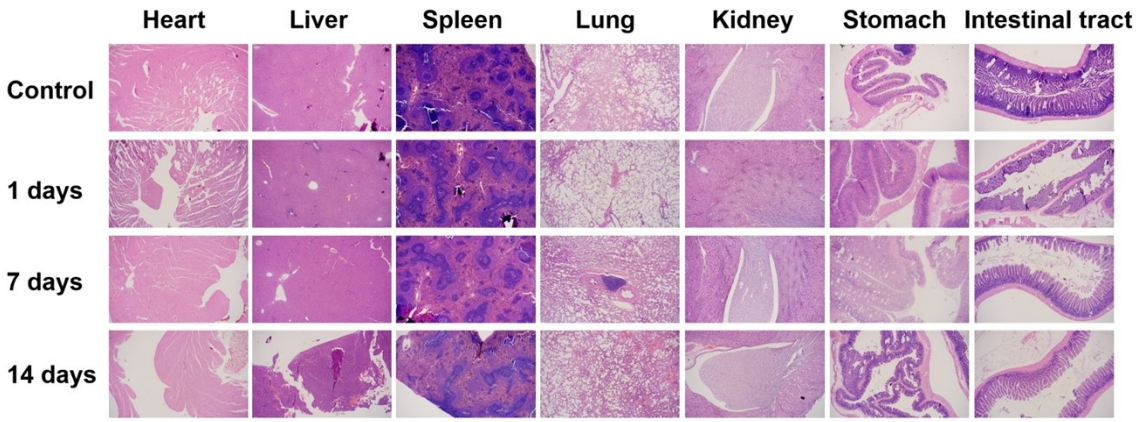

Figure S15. Hematoxylin and Eosin (H&E) staining analysis of the main organs.

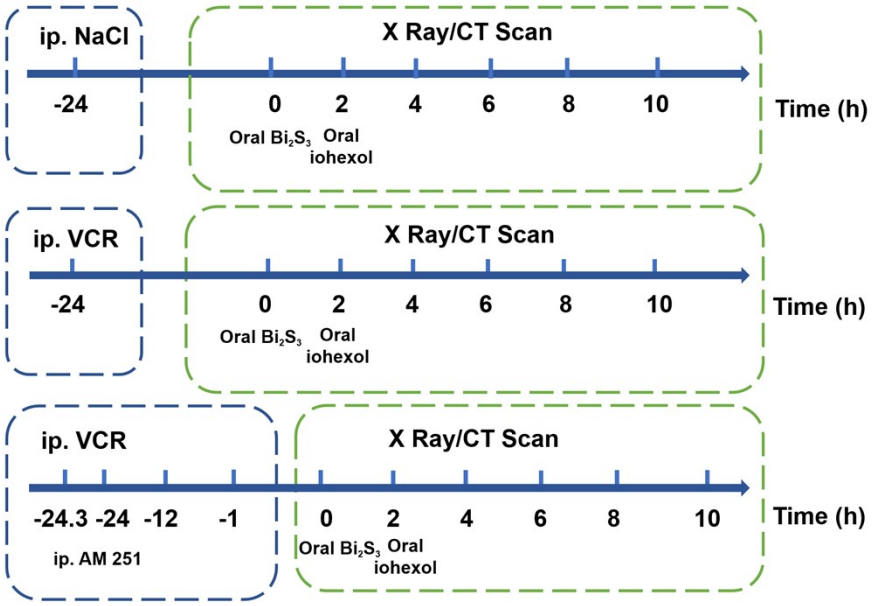

Figure S16. Schedule of various models preparation.

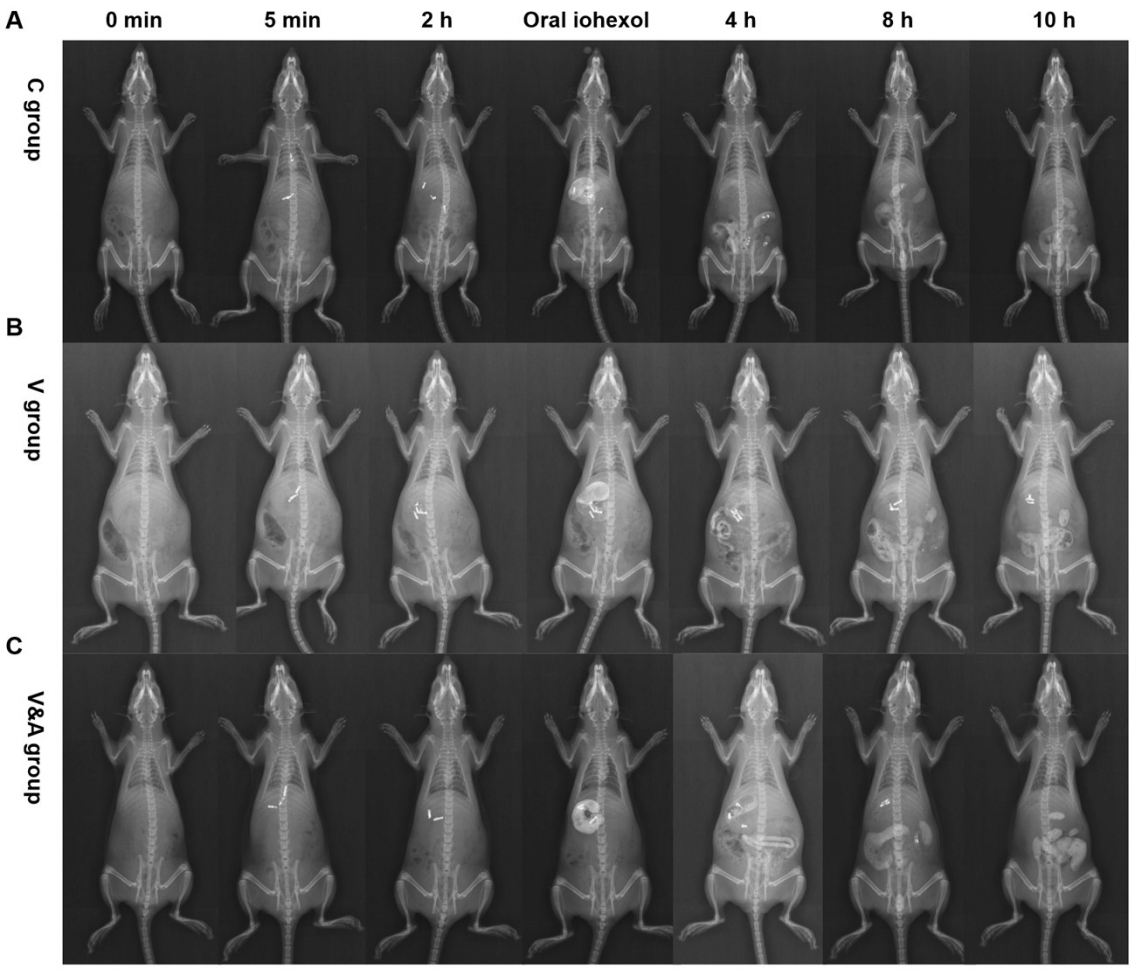

Figure S17. X-ray imaging of rats after various treatments at different time points (0 min, 5 min, 2 h, 4 h, 8 h, 10 h) in vivo. (A) control group (C group), (B) vincristine-treated group (V group), (C) vincristine & AM251 treated group (V&A group).

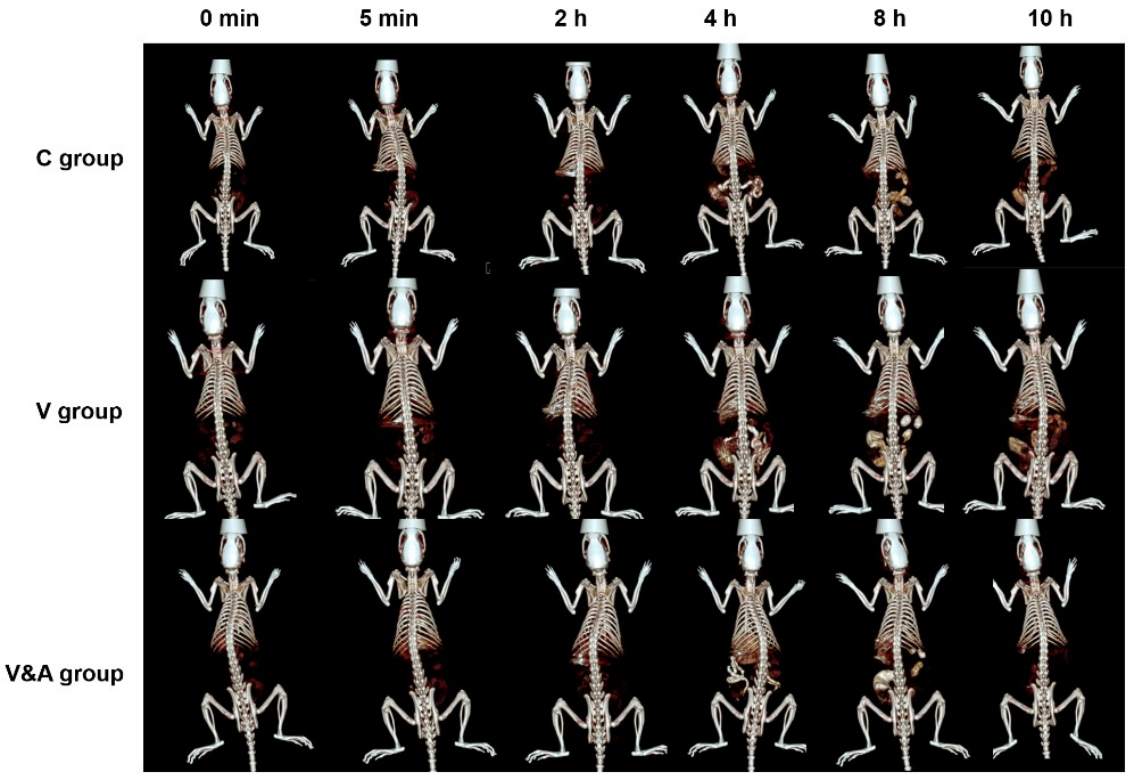

Figure S18. CT imaging of rats after various treatments at different time points (0 min, 5 min, 2 h, 4 h, 8 h, 10 h) in vivo. (A) control group (C group), (B) vincristine-treated group (V group), (C) vincristine & AM251 treated group (V&A group).

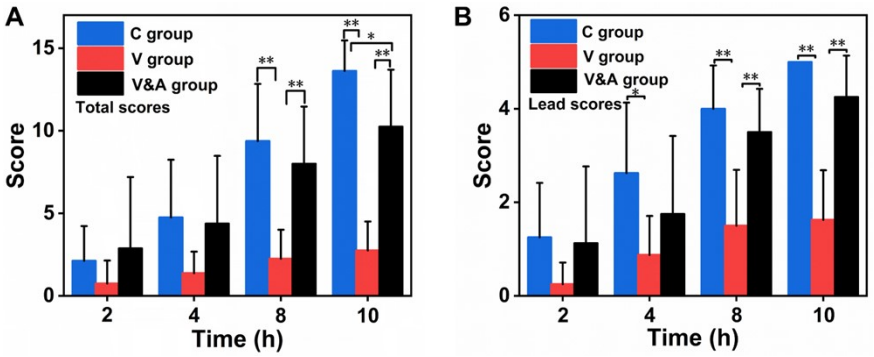

Figure S19. The total scores (A) and lead capsule scores (B) of CT imaging of rats after various treatments at different time points. These data were shown as means ± SD, n = 8, evaluated by One-way analysis of variance, *$p < 0.05$, **$p < 0.01$.

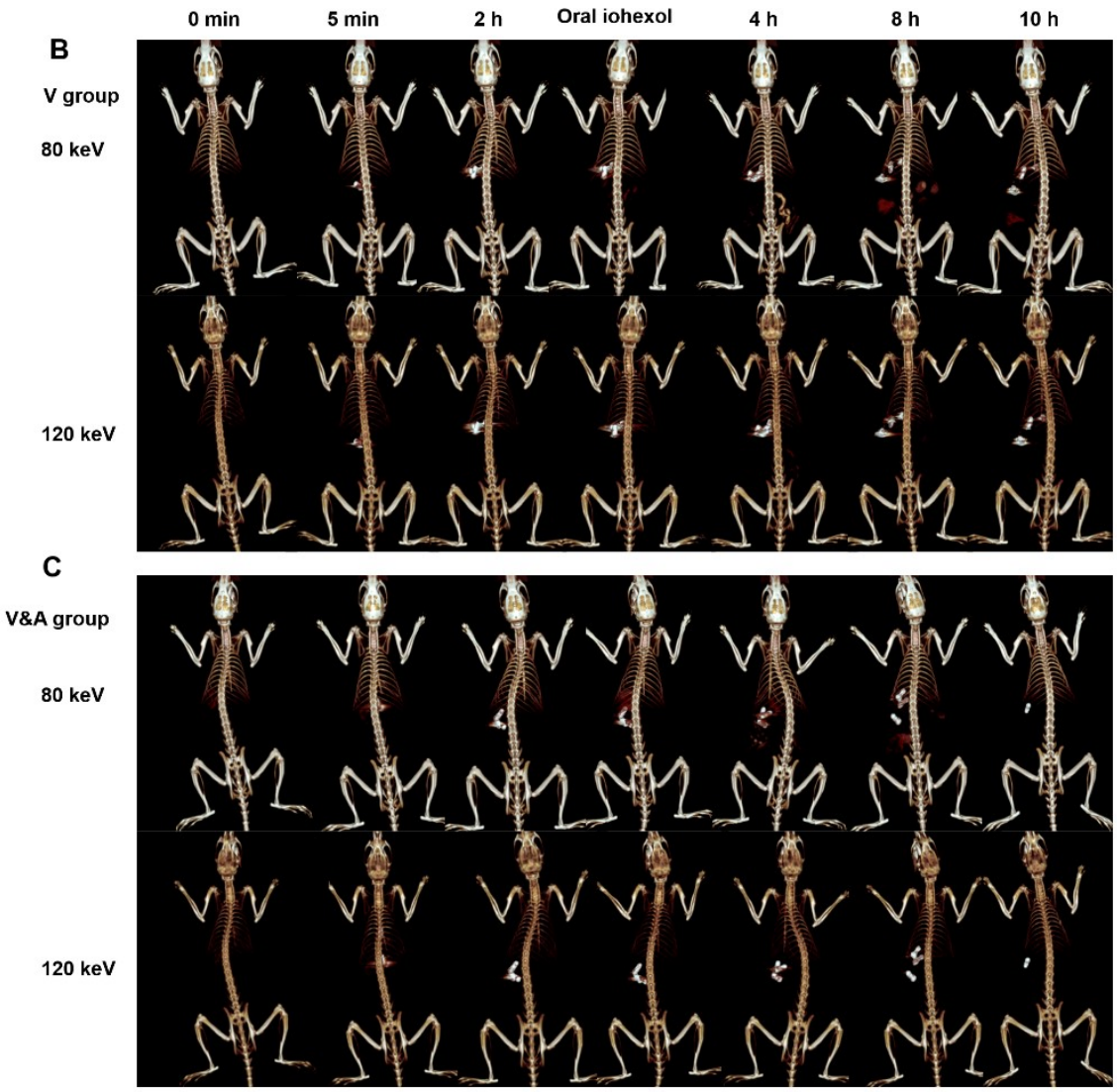

Figure S20. Three-dimensional reconstructed images of rats under different monochromatic energies after various treatments at different time points (0 min, 5 min, 2 h, 4 h, 8 h, 10 h) in vivo. (A) control group (C group), (B) vincristine-treated group (V group), (C) vincristine & AM251 treated group (V&A group).

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