Fabrication of gelatin Bi$_2$S$_3$ capsules as a highly sensitive X-ray contrast agent for gastrointestinal motility assessment in vivo†

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Tiny BaSO$_4$ rod-based X-ray imaging is the most frequently-used method for clinical diagnosis of gastrointestinal motility disorders. The BaSO$_4$ rods usually have a small size to pass through the gastrointestinal tract smoothly, but suffer from unavoidably low sensitivity. Herein, we developed Bi$_2$S$_3$ capsules as a high-performance X-ray contrast agent for gastrointestinal motility assessment for the first time. The Bi$_2$S$_3$ capsules were synthesized by the encapsulation of commercial Bi$_2$S$_3$ powder into commercial gelatin capsules and subsequent coating of ultraviolet-curable resin. The prepared Bi$_2$S$_3$ capsules showed excellent biocompatibility in vitro and in vivo and superior X-ray attenuation ability due to the large atomic number and high K-edge value of Bi. The developed Bi$_2$S$_3$ capsules can serve as a small but highly sensitive X-ray contrast agent to quantitatively assess gastrointestinal motility in a vincristine-induced gastrointestinal motility disorder model in vivo by X-ray, CT and spectral CT imaging successfully, solving the intrinsic drawbacks of clinically used BaSO$_4$.

Introduction

Gastrointestinal motility disorders are common complications of a variety of diseases, such as diabetes, obesity, hypertension, and adverse reactions during treatment with chemotherapy drugs like vincristine, which may lead to constipation or diarrhea. For instance, nearly one-third of patients receiving vincristine treatment will have symptoms such as constipation and paralytic intestinal obstruction induced by autonomic nervous system dysfunction, which can last for several months and seriously affects the patient’s quality of life and treatment compliance. Eventually, gastrointestinal motility disorders lead to the decreased absorption of nutrients and changes in the intestinal barrier function and strongly affect the normal function of the body. Thus, timely and accurate assessment of gastrointestinal function has far-reaching clinical significance.

Non-invasive imaging examinations, such as radiopaque marker tests, radionuclide scintigraphy and wireless motility capsules, have been frequently used in the diagnosis of various gastrointestinal diseases due to the advantage of non-invasiveness. Among these clinical imaging technologies, radiopaque marker tests owns the merits of simple operation, easy analysis and low cost. The use of the first radiopaque agent most likely dates back to 1896, when BaSO$_4$ was used to study gastrointestinal motility of animals and various morphologies of BaSO$_4$ agents have been developed to meet the requirement of different diseases during the past decades. Currently, for gastrointestinal motility assessment in clinic, the patients orally take 2 capsules (containing 20 BaSO$_4$ rods) after breakfast on the day of examination. Then the whole gut transit time was evaluated through observing the change of the position and the number of BaSO$_4$ rods at different time points by X-ray imaging. To satisfy the need of normal gastrointestinal transport, BaSO$_4$ rods are usually small in size. While size is a double-edged sword, which results in inherent low sensitivity of BaSO$_4$ rods and makes it difficult to definite their position in gastrointestinal tract. However, BaSO$_4$ rods have been used for gastrointestinal motility assessment for several decades, and there were few new X-ray contrast agents reported up to now. Thus, it is our great desire to develop a highly sensitive and biocompatible X-ray contrast agent for gastrointestinal motility assessment.

Besides X-ray imaging, X-ray computed tomography (CT) imaging, which is capable of three-dimensional reconstruction, is increasingly used in gastrointestinal examinations, and provides abundant diagnosis information that X-ray imaging fails to give. Especialy, the principle of spectral CT is to collect two data sets from the same anatomical location using different kilovolt peaks, possesses differentiation ability towards materials with different absorption under various X-ray energies. It can reduce image artifacts, distinguish tissue components, and improve the contrast effect of high atomic
number elements-based contrast agents to surrounding tissues, which provides a promising way to assess gastrointestinal motility. Similar to X-ray imaging, high-performance contrast agents are also the key to sensitive and accurate evaluation of gastrointestinal motility by CT and spectral CT. However, BaSO₄ and iodine-based small molecules commonly used clinically are not suitable for CT and spectral CT imaging due to the low K-edge energies of Ba and I and relatively poor X-ray attenuation ability.

Bi has the highest atom number among non-radioactive elements ($Z = 83$) and possesses an outstanding X-ray attenuation coefficient (Bi: 5.74, Au: 4.99, Pt: 4.3 cm$^2$ g$^{-1}$ at 100 keV). In addition, Bi is the cheapest element among the heavy metal elements suitable for X-ray imaging. Besides, Bi is a highly biocompatible element, and many Bi-based compounds, known as “bismuth therapy”, have served as drugs for gastrointestinal diseases for more than three hundred years. In the past decades, various Bi-based nanomaterials, such as Bi, Bi$_2$O$_3$, Bi$_2$Se$_3$, Bi$_2$S$_3$, have been developed as the new generation of X-ray contrast agents for diagnosis of various diseases. However, a Bi-based contrast agent for gastrointestinal motility assessed by X-ray, CT, and spectral CT has not been reported so far (Scheme 1).

Herein, we proposed a highly sensitive method for gastrointestinal transmission assessment using Bi$_2$S$_3$ capsules by X-ray, CT, and spectral CT imaging in vivo. The radiopaque capsules rather than water-soluble nanoparticles are needed in assessment gastrointestinal tract transmission. Commercial Bi$_2$S$_3$ powder was selected as Bi precursor due to its excellent chemical stability, high content of Bi element, good X-ray absorption capacity and low price. It was encapsulated in commercial gelatin capsules and then coated with ultraviolet-curable resin to generate the uniform Bi$_2$S$_3$ capsules. The prepared Bi$_2$S$_3$ capsules had good stability and superior X-ray (especially high-energy X-ray) attenuation ability compared to BaSO$_4$. High sensitive gastrointestinal transmission assessment by X-ray imaging was successfully achieved using Bi$_2$S$_3$ capsules, which was much more sensitive than that using BaSO$_4$. Besides, Bi$_2$S$_3$ capsules were also employed in CT and spectral CT imaging in vivo, and real-time, sensitive, and high-spatial resolution visualization of the gastrointestinal motility was realized, providing abundant three mentions information. In vitro and in vivo toxic studies proved the excellent biocompatibility of Bi$_2$S$_3$ capsules. To the best of our knowledge, it was the first time that a Bi-based contrast agent was used to evaluate gastrointestinal motility, which showed great potential as an alternative to clinic BaSO$_4$.

Results and discussion

Synthesis and characterization of Bi$_2$S$_3$ capsules

The commercial Bi$_2$S$_3$ powder with tiny size was used as the precursor for the synthesis of Bi$_2$S$_3$ capsules to achieve high imaging sensitivity (Fig. 1A and S2†). The X-ray diffraction pattern indicated the Bi$_2$S$_3$ belonged to the orthorhombic system (Fig. 1C). Despite the size of Bi$_2$S$_3$ powder showed the evident variability on micrometer scale (Fig. S3†), it was uniform on millimeter scale, which was sufficient to produce uniform Bi$_2$S$_3$ capsules (Fig. 1B). Synthesizing biomaterials with biocompatible macromolecules can improve their biosafety, so we used gelatin capsules as a carrier to prepare Bi$_2$S$_3$ capsules (Fig. 1D). The resin coating was essential to ensure the stability of Bi$_2$S$_3$ capsules in gastrointestinal tract environments. The synthesized Bi$_2$S$_3$ capsules had a uniform size with 3 mm in diameter and 8 mm in length and there were 25 mg Bi$_2$S$_3$ in each capsule. Thus, the synthesis of Bi$_2$S$_3$ capsules was extremely and reproducible.

To investigate the stability of Bi$_2$S$_3$ capsules in stimulated gastrointestinal tract environments, the Bi$_2$S$_3$ capsules were immersed into diluted HCl solution (pH = 1) and artificial small
intestinal fluid for 12 h. The morphology of the capsule had no obvious change, and there was no leakage of Bi$_2$S$_3$ power. The results indicated the Bi$_2$S$_3$ capsules could resist the gastrointestinal tract environments and keep stable for potential applications (Fig. S4†).

**X-ray, CT and spectral CT imaging in vitro**

To compare the X-ray absorbance ability of Bi$_2$S$_3$ with clinical BaSO$_4$ and iohexol *in vitro*, different suspensions were synthesized by dispersing Bi$_2$S$_3$, BaSO$_4$ and iohexol power into alginate–Ca$^{2+}$ hydrogel. In addition, BaSO$_4$ and iohexol capsules were also prepared according to the synthesis procedures of Bi$_2$S$_3$ capsules. X-ray images of various suspensions indicated their brightness increased with the increase of concentrations of radiopaque elements (Bi, Ba, and I), and the Bi$_2$S$_3$ suspension showed a higher brightness than BaSO$_4$/iohexol suspensions at an equivalent mass or molar concentrations of radiopaque elements (Fig. S5†). And the Bi$_2$S$_3$ capsule was much brighter than that of BaSO$_4$ and iohexol capsule (Fig. S6†). It should be noted that there was serious interference from food in gastrointestinal tract imaging, and iohexol was used to visualize the gastrointestinal tract profile sometimes, so the imaging ability of the capsules should also be evaluated in the presence of food or iohexol. These capsules all showed much higher brightness than food or iohexol solution visually, and the boundary between capsules and surrounding food or iohexol can be seen obviously (Fig. S7†). Quantitative analysis indicated Bi$_2$S$_3$ capsules exhibited the highest brightness among them. These results indicated Bi$_2$S$_3$ capsules had great potential in serving as a superior X-ray contrast agent.

In CT imaging, with the increasing concentrations of radiopaque elements, the CT images of Bi$_2$S$_3$ suspensions were brighter than the corresponding images of BaSO$_4$ and iohexol suspensions at various tube voltages (80, 100, 120, and 140 kV). Meanwhile, the Hounsfield unit (HU) values linearly increased under the same voltage for Bi$_2$S$_3$, BaSO$_4$ and iohexol suspensions and capsules in a concentration dependent manner and the slope discrepancy among Bi$_2$S$_3$, BaSO$_4$ and iohexol became more and more distinct along with the increasing tube voltage from 80 to 140 kV (Fig. 2A–D, S8 and S9†). The CT imaging ability of Bi$_2$S$_3$ capsules in food and iohexol were also investigated, and the results confirmed that Bi$_2$S$_3$ capsules had the best CT imaging performance even in the high background induced by food and iohexol (Fig. S10A and S11†). In order to study the feasibility of spectral CT imaging using Bi$_2$S$_3$, monochromatic images of Bi$_2$S$_3$, BaSO$_4$ and iohexol suspensions were obtained (Fig. 3A–C and S12†). Under each X-ray energy, there is a linear relationship between HU value and concentration of contrast agents. At low X-ray energies (40–50 keV), the HU values of BaSO$_4$ and iohexol suspensions were higher than those of Bi$_2$S$_3$ suspensions. As the tissues were mainly composed of low atomic number elements, they showed similar X-ray absorption properties to BaSO$_4$ or iohexol and also have a relatively high X-ray absorption at low X-ray energies. Hence it is useless to perform spectral CT imaging using BaSO$_4$ or iohexol as contrast agents at low X-ray energies. When the energy is larger than about 60 keV, the HU values of Bi$_2$S$_3$ suspensions became higher than those of BaSO$_4$ and iohexol suspensions, and the slope (HU value per unit concentration) difference between Bi$_2$S$_3$ suspensions and BaSO$_4$/iohexol suspensions becomes more and more obvious when monochromatic energy increased.

**Fig. 1** Characterization of Bi$_2$S$_3$ powder and capsules. (A) The photo of commercial Bi$_2$S$_3$ powder. (B) The photo of dispersed Bi$_2$S$_3$ power in millimeter scale. (C) X-ray diffraction pattern of Bi$_2$S$_3$ powder. (D) The photo of empty gelatin capsule (upper panel) and Bi$_2$S$_3$ capsules (lower panel).

**Fig. 2** HU curves and CT images of Bi$_2$S$_3$, BaSO$_4$ and iohexol suspensions with different concentrations of radiopaque elements (0.0125, 0.025, 0.05, 0.1, and 0.2 M Bi, Ba or I) at (A) 80 kV, (B) 100 kV, (C) 120 kV, (D) 140 kV.
Compared with the sharp decrease of the HU value of BaSO₄ and iohexol suspensions with the increase of monochromatic energy, Bi₂S₃ suspensions exhibit relatively stable HU value due to the high K-edge energy of Bi (91 keV) (Fig. 3D). These results indicated Bi₂S₃ exhibited excellent spectral CT imaging ability at high X-ray energies. Then we evaluated the spectral CT imaging ability of Bi₂S₃ capsule. Similar to Bi₂S₃ suspension, Bi₂S₃ capsule showed better CT imaging performance when the energy increased from 80 keV to 160 keV, and the energies were higher, the performance was better (Fig. 4). In the presence of food, three kinds of capsules all showed a good contrast effect, and the boundary between capsules and food was very clear, but a quantitative analysis indicated the HU value of Bi₂S₃ capsule was the highest (Fig. S10B†). In the presence of iohexol, it is hard to distinguish capsules from iohexol only if the concentration of iohexol was very low (5 mg mL⁻¹) at low X-ray energies. However, when the X-ray energies were larger, the contrast of HU values of capsules and iohexol solution became significant, and the boundary between them can be seen obviously (Fig. S13†). It should be noted the size of Bi₂S₃ capsule kept the same in spectral CT images at various X-ray energies while the size of BaSO₄ capsule became smaller along with the increase of X-ray energies. Besides, the HU values of Bi₂S₃ capsule were higher than those of BaSO₄ capsule at various X-ray energies. These results demonstrated Bi₂S₃ capsule can serve as a spectral CT imaging contrast agent with high sensitivity, which was capable of minimizing the interference of surrounding substance.

**Cytotoxicity assessment**

The cytotoxicity of Bi₂S₃ capsules was evaluated by a standard MTT test. Firstly, Bi₂S₃ capsules were dispersed in various media (ultrapure water, phosphate buffered saline (PBS) solution (pH = 7.4) and diluted hydrochloric acid (pH = 1)) for 24 h, and the leaching solutions were acquired by removing the capsules. Then, 3T3-L1 cells were incubated with various leach solutions for 12 h and the cell viabilities were determined. The results indicated Bi₂S₃ capsule leach solutions had little influence on the proliferation of 3T3-L1 cells, and the cell viability remained above 80% (Fig. S14†), indicating the low cytotoxicity of Bi₂S₃ capsules.

**In vivo toxicity of Bi₂S₃ capsule**

To investigate the in vivo toxicity of Bi₂S₃ capsules, hematoxylin and eosin (H&E) staining analysis of the main organs (heart, liver, spleen, lung, kidney, and the intestinal tract) at different time points was carried out after oral administration of three Bi₂S₃ capsules (Fig. S15†). H&E staining results demonstrated there were no obvious histopathological damages in major organs of rats after the treatment of Bi₂S₃ capsules, further proving their good biocompatibility.

**Gastrointestinal transmission assessment by X-ray imaging in vivo**

Based on the excellent X-ray absorption ability of Bi₂S₃ capsules, we investigated the feasibility of gastrointestinal transmission assessment using Bi₂S₃ capsules by X-ray imaging on vincristine-induced gastrointestinal motility disorder models. Paralytic ileus caused by vincristine can be prevented/treated by CB1 antagonists, such as AM251.③ The rats were divided into three groups including a control group, vincristine-treated group and vincristine & AM251-treated group (n = 8 in each group). After oral administration of three Bi₂S₃ capsules, the X-ray imaging was carried out at different time points (0 min, 5 min, 2 h, 4 h, 8 h, and 10 h) (Fig. 5A–C). For all the groups of rats, three Bi₂S₃ capsules can be seen clearly in esophagus and stomach after the administration of capsules in X-ray imaging, and then the capsules moved into small intestinal gradually. At 2 h, the rats were orally

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**Fig. 3** HU curves and spectral CT images of Bi₂S₃, BaSO₄ and iohexol suspensions at different concentrations of radiopaque elements (0.0125, 0.025, 0.05, 0.1, and 0.2 M Bi, Ba or I) at (A) 40 keV, (B) 80 keV, (C) 120 keV. (D) Spectral CT HU curves and images of Bi₂S₃, BaSO₄ and iohexol (0.1 M Bi, Ba or I) at different monochromatic energies.

**Fig. 4** Spectral CT images and HU values of full of Bi₂S₃, BaSO₄ and iohexol capsules.
administrated with iohexol to visualize the gastrointestinal tract profiles, benefiting the determination of the location of Bi$_2$S$_3$ capsules. For the control group, the capsules were mainly located in small intestinal before 8 h, and then moved into cecum and colon at about 8 h. The majority of capsules can be excreted from the body at 10 h. In contrast, for the vincristine-treated group, the movement speed of capsules slowed down obviously, and the majority of capsules were still located into small intestinal at 10 h due to the weakened gastrointestinal motility. While the metabolism of the capsules in gastrointestinal tract became normal after vincristine-treated rats were treated with AM251, as shown in the X-ray imaging. In addition, BaSO$_4$ capsules were also used to assess gastrointestinal motility in the rats with various treatments. However, the contrast effect of BaSO$_4$ capsules in X-ray imaging was much worse than that of Bi$_2$S$_3$ capsules, which demonstrated Bi$_2$S$_3$ can serve as an excellent alternative X-ray contrast agent to BaSO$_4$ (Fig. S17†).

Then we quantitatively assessed the gastrointestinal motility based on a standard rating scale (Table 1), and two kinds of scores (total score and lead capsule score) were calculated for the assessment. The three capsules are given numerical scores based on their position in the gastrointestinal tract, and the total score is calculated by adding the score of each capsule (if all are excreted, the maximum is equal to 15). The lead capsule score was calculated based on its position in the gastrointestinal tract according to the rating scale (if it is excreted, the maximum is equal to 5). As shown in Fig. 6A, the average total scores of the vincristine group were 7.8 times at 4 h, 4.6 times at 8 h and 3.9 times at 10 h lower than those of the control group, respectively. After treated by AM251, the average total scores of vincristine & AM251 group increased by 4.8 times at 4 h, 3.2 times at 8 h and 2.8 times at 10 h compared with those of the VCR group, which indicated the gastrointestinal motility disorder caused by vincristine was significantly restored by AM251.

Besides, the lead capsule score was also used to evaluate the gastrointestinal motility. The lead capsules score of vincristine group were 2.4 times at 4 h, 2.7 times at 8 h, and 2.3 times at 10 h lower than those of the control group, respectively (Fig. 6B). The lead capsules in vincristine group only reached the distal small intestine area, while most of lead capsules in the control group were excreted at 10 h after oral administration. The lead capsules scores of vincristine & AM251 group were 1.6 times at 4 h, 2.3 times at 8 h and 2 times at 10 h higher than those of vincristine group. The lead capsules in the gastrointestinal tract were either in the descending colon or excreted at 10 h due to the recovery of gastrointestinal motility function.

**Gastrointestinal transmission evaluation by CT and spectral CT imaging in vivo**

Then the gastrointestinal motility assessment was evaluated by CT and spectral CT imaging using Bi$_2$S$_3$ capsules. The metabolism behavior of Bi$_2$S$_3$ capsules in the gastrointestinal tract of rats in various groups can be monitored in three dimensions clearly, providing more abundant diagnosis information. The gastrointestinal motility disorder and function recovery can be diagnosed by the visualized CT images and rating scores (Fig. S18 and S19†), and the results were consistent with those obtained from X-ray imaging. Besides, the spectral CT imaging

**Table 1** Bi$_2$S$_3$ capsule position score. Lead capsule refers to the most distally located capsule. Total score is determined by summing the scores of three capsules.

| Position        | Stomach | Proximal small intestine | Distal small intestine          |
|-----------------|---------|--------------------------|---------------------------------|
| Score           | 0       | 1                        | 2                               |
| Position        | Cecum   | Colon                    | Excretion of the gastrointestinal tract |
| Score           | 3       | 4                        | 5                               |

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with the charming advantage of distinguishing the materials by different atomic numbers was also carried out (Fig. 7A–C and S20†). In common CT imaging or spectral CT imaging at low monochromatic energies, food, faeces, iohexol and other tissues all can be seen besides Bi$_2$S$_3$ capsules, and which resulted in troublesome interferences for the clear visualization of Bi$_2$S$_3$ capsules. While in spectral CT imaging at high monochromatic energies, the CT signal of other subjects decreased significantly, even disappeared. In contrast, Bi$_2$S$_3$ capsules still kept the constant strong brightness in spectral CT imaging regardless of the monochromatic energies, so the Bi$_2$S$_3$ capsules can be distinguished from the background very obviously at high monochromatic energies. Combined spectral CT imaging of Bi$_2$S$_3$ capsules at low and high monochromatic energies, the location information of Bi$_2$S$_3$ capsules and anatomical information of surrounding tissues can be obtained high sensitively and accurately. Therefore, the developed Bi$_2$S$_3$ capsules can serve as an excellent multifunctional X-ray contrast agent for gastrointestinal motility assessment based on X-ray, CT and spectral CT.

Conclusions

In summary, we reported the facile fabrication of Bi$_2$S$_3$ capsules as a high-performance X-ray contrast agent for gastrointestinal motility assessment for the first time. Bi$_2$S$_3$ capsules were synthesized by encapsulating commercial Bi$_2$S$_3$ powder into gelatin capsules, followed by the coating of ultraviolet-curable resin. In the harsh environment of the gastrointestinal system, the synthesized Bi$_2$S$_3$ capsules can still keep intact morphology. The prepared Bi$_2$S$_3$ capsules showed superior X-ray absorption ability than BaSO$_4$ capsules at various conditions in X-ray and CT imaging in vitro. In particular, the large atomic number and high K-edge value of Bi ensure the outstanding contrast performance of Bi$_2$S$_3$ capsules at high monochromatic energies. The cellular studies proved the low cytotoxicity of Bi$_2$S$_3$ capsules, and the in vivo toxicity evaluation also confirmed the good biocompatibility of Bi$_2$S$_3$ capsules. Taking the vincristine-induced gastrointestinal motility disorder model as an example, the developed Bi$_2$S$_3$ capsules were successfully employed to quantitatively and sensitively assess gastrointestinal motility through X-ray, CT and spectral CT imaging in vivo, and the imaging performance of Bi$_2$S$_3$ capsules was much better than that of BaSO$_4$ capsules. Our study demonstrated the feasibility of a highly sensitive gastrointestinal motility assessment using a Bi-based contrast agent with great clinical translation potential, which can serve as an excellent alternative contrast agent to clinically used BaSO$_4$.

Author contributions

S.-K. Sun and X.-J. Zhang supervised the project. Y. Wen and W. Zhu performed the experiments. Y. Wen principally wrote the manuscript. All authors proof-read, provided comments, and approved the final version of this manuscript.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (21874101, 21934002, and 82071982) and the Natural Science Foundation of Tianjin City (19JCYJC63700) and Young Elite Scientists Sponsorship Program by Tianjin (TJSQNTJ-2018-08).

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