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Non-toxic lead sulfide nanodots as efficient contrast agents for visualizing gastrointestinal tract

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ABSTRACT

Non-invasive imaging of gastrointestinal (GI) tract using novel but efficient contrast agents is of the most important issues in the diagnosis and prognosis of GI diseases. Here, for the first time, we reported the design and synthesis of biothiol-decorated lead sulfide nanodots, as well as their usages in functional dual-modality imaging of GI tract in vivo. Due to the presence of glutathione on the surface of the nanodots, these well-prepared contrast agents could decrease the unwanted ion leakage, withstand the harsh conditions in GI tract, and avoid the systemic absorption after oral administration. Compared with clinical barium meal and iodine-based contrast agents, these nanodots exhibited much more significant enhancement in contrast efficiency during both 2D X-ray imaging and 3D CT imaging. Different from some conventional invasive imaging modalities, such as gastroscope and enteroscope, non-invasive imaging strategy by using glutathione modified PbS nanodots as contrast agents could reduce the painfulness towards patients, facilitate the imaging procedure, and economize the manipulation period. Moreover, long-term toxicity and bio-distribution of these nanodots after oral administration were evaluated in detail, which indicated their overall safety. Based on our present study, these nanodots could act as admirable contrast agents to integrate X-ray imaging and CT imaging for the direct visualization of GI tract.

1. Introduction

Digestive diseases such as gastrointestinal (GI) tumors, intestinal malformation, esophageal varices, and unidentified GI hemorrhage are very common in recent clinical practices [1–3]. In terms of facile imaging process, no tissue damage, and painlessness towards patients, non-invasive imaging has played an important role in the diagnosis, treatment planning, and prognosis of GI diseases [4–6]. Among all these imaging modalities, 2D X-ray imaging and 3D CT imaging with deep tissue penetration, cost effectiveness, as well as remarkable resolution usually provide clinicians with powerful information in the visual representations of GI anatomy and pathology [7–10].

To afford precise description of digestive disease status, orally or rectally administrated contrast agents are highly essential [11–14]. Nowadays, barium sulfate in suspension is frequently used as radiocontrast agents for 2D X-ray GI imaging in clinic. However, unknown median lethal dose (LD_{50}) value and concomitant false positive results may seriously limit its clinical universality [7]. To better differentiate the details of imaging organs with other similar tissues, iodine-based organic small molecules such as meglumine diatrizoate have been well synthesized and applied as contrast agents for 3D CT GI imaging [11]. Although promising, only large doses of these agents can achieve adequate contrast enhancement due to the limited efficiency in absorbing X-rays of iodine. In addition, these iodinated molecules usually cause grievous iodine hypersensitivity reaction in patients during the imaging procedure. These intrinsic shortcomings significantly restrict the practical usages of barium meal and iodinated molecules as broad-spectrum contrast agents of GI tract. More importantly, information obtained from single-modality imaging can not satisfy the higher

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requirement for the diagnosis, prognosis, and further medical treatment of digestive diseases due to the exhibited default rooted in each imaging technique [15–19]. Therefore, efficient multi-modality contrast agents are highly desired in attempting to achieve more complementary and accurate information of GI diseases.

Recently, novel nanomaterials used as X-ray/CT contrast agents have attracted much more attention among all these imaging modalities [20–35]. To highly meet the demand of current clinical expectation, nanoparticulate contrast agents adapted for GI imaging must be rationally designed by the following criteria: (1) significant contrast enhancement after oral or rectal administration; (2) excellent stability in harsh GI conditions; (3) low systemic toxicity both in vitro and in vivo; as well as (4) cost-effective and non-complicated routes to prepare these contrast agents [36–40]. Towards this goal, several nano-sized contrast agents for GI X-ray/CT imaging have been fabricated by our and other groups, such as lanthanide-based nanoprobes, tantalum powders, iodine-based nanoparticles, tungsten oxide nanosheets, and Bi_2S_3@SiO_2 nanorods [21,41–44]. Even though these findings exhibit imaging flexibilities in the lab or clinical setting, several inherent disadvantages of these contrast agents have thoroughly confined their usages in GI imaging. For example, some lanthanide-doped nanoprobes may induce intracellular ATP deprivation and a serious decrease in cellular viability after long-term incubation [45,46]. Limited imaging efficacy of iodine-based agents cannot provide enough contrast enhancement at low concentrations [47,48]. Unclear toxicity of Ta powders induces the translation of Ta-based contrast agents from the lab design to the clinical usage impossible [21]. Otherwise, multi-step preparations of tungsten oxide nanosheets and Bi_2S_3@SiO_2 nanorods are highly dependent on the experimental skills.

Similar with element bismuth, lead also holds a high atomic number and an admirable X-ray absorption under clinical voltages [20]. With this speculation in mind, we prepared biothiol-decorated lead sulfide nanodots here and then for the first time used them as efficient non-leakage X-ray/CT contrast agents for GI imaging. As expected, glutathione coated on the surface of the nanodots could extremely decrease the unwanted leakage of lead ions, withstand the harsh conditions of GI tract, and decrease unnecessary absorption by GI tissues. With great colloid stability, low cytotoxicity, and neglectable hemolysis, these contrast agents exhibited much more significant contrast enhancement in both 2D X-ray imaging and 3D CT imaging than routinely used barium meal and meglumine diatrizoate, respectively. More importantly, our imaging strategy using these nanodots as contrast agents could sufficiently overcome the limitations of traditional invasive imaging modalities. In addition, bio-distribution and long-term toxicity of these nanodots after oral administration were investigated, indicating their overall safety in vivo. Taking together, these well-prepared biothiol-decorated lead sulfide nanodots exhibited more promise for pre-clinical and clinical GI imaging paradigm.

2. Materials and methods

2.1. Chemical and materials

Lead acetate ($Pb(CH_3COO)_2 \cdot 3H_2O$), sodium sulfide ($Na_2S$), L-glutathione reduced (GSH), diethylene glycol (DEG), and polyacylic acid (PAA) were purchased from Sigma-Aldrich. Chloral hydrate, thiourea (TU), and sodium hydroxide (NaOH) were achieved from Aladdin Reagent. Other reagents were obtained from Beijing Chemicals. All chemical agents were of analytical grade and used directly without further purification. Water throughout all experiments was obtained via a Milli-Q water system.

2.2. Synthesis of GSH-modified PbS nanodots

GSH-modified PbS nanodots (denoted as GSH-PbS NDs) were prepared via a facile one-pot route. Typically, aqueous solutions containing lead acetate (0.1 M, 10 mL) and GSH (0.1 M, 20 mL) were added to a three necked flask with deionized water (210 mL) under nitrogen atmosphere. The pH value was adjusted with NaOH (1 M) to 10 and the temperature of above system was adjusted to 50 °C. Aqueous solution containing Na_2S (0.1 M, 10 mL) was added slowly to result in production with dark color. 15 min later, resultant product was naturally cooled down to room temperature. The suspension was separated via centrifugation, collected after washing, and achieved via freeze-drying. For the preparation of nude PbS NDs, GSH molecules were not involved in the typical synthesis.

2.3. Synthesis of PbS colloidal nanoparticles

PbS nanoparticles with an average diameter of 160 nm were prepared via a high-temperature polyol-mediated reaction. Typically, TU (4 mmol) was added into DEG (10 mL) to result in a TU/DEG stock solution. Upon nitrogen atmosphere, above solution was heated to 100 °C and maintained for another 1 h. DEG solution (6 mL) containing PAA (6 mmol) and lead acetate (0.4 mmol) was heated to 210 °C under a protective nitrogen atmosphere. Above stock solution (2 mL) was then added rapidly into this hot mixture. The resulted solution was further heated at 210 °C for 10 min. The products were cooled to room temperature naturally. After careful washing with ethanol and water, these nanoparticles were finally achieved via freeze-drying.

2.4. Leaching study of Pb^{2+} from GSH-PbS NDs

Aqueous solution containing GSH-PbS NDs (10 mg/mL) was taken into a dialysis bag and dialyzed against simulated gastric juice or simulated intestinal fluid. 48 h later, above solutions were collected. Inductively coupled plasma mass spectrometry (ICP-MS) was then used to determine the content of Pb^{2+} ions.

2.5. Cell cultures

Hela cells and Caco-2 cells were supplied by ATCC (American Type Culture Collection). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing penicillin (100 U/mL), streptomycin (100 U/mL), and 10% fetal bovine serum (FBS) in a humidified incubator at 37 °C and 5% CO_2. Cells were harvested by the use of trypsin and were re-suspended in fresh complete medium before plating.

2.6. Cytotoxicity

Hela cells were cultured in 96-well plates as a density of 5×10^3 per well for 12 h to allow the cellular attachment. Subsequently, nanodots were added into above culture medium. At the end of incubation periods, medium containing nanodots was removed, and the cell samples were treated with MTT for another 4 h. To dissolve the formazan crystals, dimethyl sulfoxide (DMSO) was added. Bio-Rad model-680 microplate reader was used to measure the absorbance at a wavelength of 570 nm. The absorbance of nanodots at different concentrations was measured as the deductible background. Several independent replicates were done for each group and percent viability was normalized to the cell viability in the absence of GSH-PbS NDs. In addition, Caco-2 cells were cultured in 96-well plates as a density of 2×10^3 per well for 24 h to allow the cellular attachment. Two days later, nanodots with