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The ratiometric fluorescence nanoparticle based on SiRB for pH detection of tumor

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ABSTRACT

Tumor pH detection and pH value change monitoring have been of great interest in the field of nanomedicine. In this study, a pH-sensitive near-infrared fluorescence probe SiRB (Si-rhodamine and Boronic acid group) was synthesized by introducing a boronic acid group into the silicon rhodamine structure. ICG (Indocyanine green) as the fluorescence internal standard and SiRB were loaded into PLGA (poly lactic-*co*-glycolic acid) to form PLGA-SiRB-ICG nanoparticle. The experiments showed that the size of the nanoparticle was about 90 nm, which can reach tumor passively by enhancing permeability and retention effect. PLGA in the acidic environment will accelerate the release of cleavage, and the fluorescence ratio of the two probes can reflect the specific pH value in the tumor. The results indicated that the nanoparticle could quantitatively measure the pH value of the tumor site, which is expected to be used in tumor research and treatment.

1. Introduction

Tumor microenvironment is a complex internal environment composed of tumor cells, interstitial cells and extracellular matrix (Gandellini et al., 2015; Hui and Chen, 2015; Wu and Dai, 2017; Alkatout et al., 2017). The characteristics of tumor microenvironment, such as high interstitial fluid pressure, hypoxia, and low extracellular pH, are closely related to the various prognostic factors that control the tumor growth and metastasis (Yang and Yu, 2015; Joyce, 2005). Studies have shown that the tumor acidic environment is caused by anaerobic glycolysis and acidic substance removal disorder (Zhao et al., 2016). Not only would it increase the potential of tumor migration and invasion, but also obstruct the activities of p53 and p-glycoprotein, leading to the MDR (multidrug resistance) in chemotherapy (Florence et al., 2015; Yan and Jurasz, 2016). Therefore, developing a rapid and accurate measurement method of tumor microenvironment pH is conducive to the diagnosis and prognosis of the tumor.

With the development of fluorescent imaging technology and its application in medical imaging, more and more fluorescent probes are synthesized for the detection of small molecule substances. For instance, Wang et al. reported a FRET-based fluorescent polymer dots for the ratiometric imaging of lysosomal HClO in living cells; Chen et al. designed a fluorescent sensor for sensitive and selective detection of copper(II) ion and sulfide anion (Wang et al., 2017; Chen et al., 2016). Among the methods of detecting tumor pH, one way is to connect the fluorophore to the quencher via the hydrazone bonds, which would break in acidic environment to control the fluorescent switch (Li et al., 2017). The drawback of this way is that the hydrazone bonds may rupture during the blood circulation and cause the fluorescence emission before reaching the tumor site. There is also a strategy is adding electron-withdrawing groups to the fluorescent groups, to control the fluorescence emission by PET (photoelectron transfer effect) (Lau et al., 2014). The disadvantages of this strategy are that the sensitivity of the fluorescent switch is low and the electron-withdrawing groups cannot completely inhibit the emission of fluorescence. Moreover, these methods can only compare the level of tumor pH, but cannot measure the specific value of pH. So it is urgently needed to develop an accurate and easy-to-operate method for pH imaging.

As a novel silicon-containing rhodamine derivatives, SiR (Si-rhodamine) has received extensive attention recently. The reason is that SiR has not only excellent photophysical properties and biocompatible properties, but also the excitation and emission wavelengths in the

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near-infrared region (Tao et al., 2017; Zhu et al., 2015). So it is particularly suitable for tumor tissue imaging. What is more, the spirocyclization of SiR is an attractive strategy for designing NIR (near infrared) fluorescent probes to detect some ions by a reversible ring-opening process (Zhu et al., 2015; Wang et al., 2012). Following this design principle, some SiR-based fluorescent dyes have been exploited for imaging Hg^{2+}, OH^{-1} and the proteins of interest from living cells. As a promising recognition group, boronic acid participates in such an equilibria: in a neutral or mildly basic environment, it binds with the substances containing hydroxyl groups and forms the corresponding boronate which can be reversibly hydrolyzed to boronic acid under acidic conditions (Dervisevic et al., 2017; H.S et al., 2017). Herein we designed and synthesized a SiR-based spirocyclic derivative SiRB (Si-rhodamine and boronic acid), in which a boronic acid group was introduced as a pH-sensitive group. SiRB displayed the reversible H^+-triggered ring-opening process of the corresponding spirocyclic structures accompanied by the remarkable chromogenic and fluorogenic changes. Furthermore, SiRB probe showed the prominent fluorescence changes in the pH range from 7.6 to 5.8, demonstrating an ideal suitability for specific labeling of acidic microenvironment of tumor and tracking the pH changes.

As a biodegradable polymer organic compound, PLGA (poly lactic-*co*-glycolic acid) has good biocompatibility, non-toxicity, good encapsulation and film-forming properties. And it is widely used in pharmaceutical, medical engineering materials and modern industry as a pH-sensitive polymer material (Gu et al., 2015; Guimaraes et al., 2015; Kanamala et al., 2016). In this work, a PLGA-based nanoparticle is fabricated for ratiometric fluorescence imaging and pH detection. It is found that two types of NIR dyes, SiRB and ICG (indocyanine green), can be effectively encapsulated into PLGA via hydrophobic interaction and induce the self-assembly of PLGA to form polymer-dye nanoparticles, with ICG, whose absorbance and fluorescence are inert to pH change, serving as the internal reference item (Kim et al., 2015; Ruhm et al., 2014). The pH-responsive dye SiRB could act as a pH indicator under ratiometric fluorescence imaging. The accurate detection of tumor pH by fluorescence imaging was realized after intravenous injection of this nanoparticle. The gradual acidification of the tumor microenvironment during the tumor growth, as well as the instant tumor pH changes upon injection of external buffers, was vividly observed by this method.

2. Materials and methods

2.1. Materials

3-Bromo-*N*,*N*-dimethylaniline, 2-bromophenylboronic acid (97%), *N*-butyldi ethanolamine (98%) and indocyanine green (ICG) were purchased from Aladdin. poly lactic-*co*-glycolic acid (PLGA, 99%) was obtained from Fu Zhong Pharmaceutical Technology Co., Ltd (Beijing, China). N-Butyl lithium, sec-butyllithium, heptane were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Tetrahydrofuran (THF), methylene chloride and acetic acid were purchased from Fuyu Fine Chemical Co., Ltd (Tianjin, China). Other reagents were acquired from Sigma-Aldrich. All the reagents were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Synthesis of SiRB

2.2.1.1. Synthesis of 4,4′-methlenebis (3-bromo-N,N-dimethylaniline) (compound 1). A solution of 3-bromo-*N*,*N*-dimethylaniline (25 mmol) in AcOH (80 mL) was added to 37% formaldehyde (125 mmol), and the mixture was stirred at 85 °C for 90 min. After cooling to room temperature, the reaction mixture was carefully neutralized with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 for three times. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by silica gel column chromatography with petroleum ether/dichloromethane (2/1, v/v) as eluent. The pure compound 1 was a white solid (yield: 71%).

2.2.1.2. Synthesis of SiX (Si-xanthone). The compound 1 (5 mmol) and anhydrous THF (tetrahydrofuran) (20 mL) were added to a pre-dried flask flushed with nitrogen. The solution was cooled to −78 °C, 1.3 M s-BuLi (sec-butyllithium) (10.7 mL) was added, and the mixture was stirred for 30 min. At the same temperature, a solution of $SiMe_2Cl_2$ (10 mmol) in anhydrous THF (10 mL) was slowly added, and the mixture was slowly warmed to room temperature, then stirred for 3 h. The reaction was quenched by the addition of 2 M hydrochloric acid. Then the mixture was neutralized with $NaHCO_3$ solution, and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude was used without further purification. $KMnO_4$ solution (15 mmol) was added to the crude dissolved 50 mL acetone at 0 °C in small portions over a period of 1 h with vigorous stirring. The mixture was stirred for another 1 h at the same temperature, then diluted with CH_2Cl_2 (50 mL), filtered through paper filter and evaporated to dryness. The residue was purified by column chromatography on silica gel to give pure SiX as a yellow solid (yield: 64%).

2.2.1.3. Synthesis of Br-B(2-(2′-bromophenyl)-6-butyl dioxazaborocan). *N*-butyldiethanolamine (20 mmol) was added to a suspension of 2-bromophenylboronic acid (20 mmol) in anhydrous toluene (30 mL). The mixture was heated at 50 °C for 2 h. After cooling to room temperature, the toluene was evaporated under reduced pressure. The remaining clear colorless crude oil was treated with heptane to remove the residual toluene. The resulting suspension was allowed to stand at room temperature overnight. The precipitated solid was collected by filtration, washed with heptane, and dried overnight to give the title compound Br-B as a white solid (yield: 58%).

2.2.1.4. Synthesis of SiRB. To a dried flask flushed with nitrogen, compound Br-B (1.0 mmol) and anhydrous THF (10 mL) were added. The solution was cooled to −78 °C, 1.6 M n-BuLi (n-Butyllithium) (0.75 mL) was added over a period of 10 min and the mixture was stirred for further 20 min. At the same temperature, a solution of SiX (1.0 mmol) in anhydrous THF (10 mL) was slowly added. The mixture was allowed to stir for 20 min at −78 °C, then allowed to warm to room temperature gradually. After stirring for further 1 h, 6 M HCl solution (10 mL) was added and the mixture was stirred for an additional 30 min. The resulting blue solution was neutralized with $NaHCO_3$ solution, and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel to give SiRB as a light blue solid (yield: 52%).

2.2.2. Synthesis of PLGA-ICG-SiRB

The PLGA-ICG-SiRB nanocomposites were obtained by self-assembly and subsequently loaded into PLGA nanoparticles. Briefly, 1 mg synthesized SiRB, 0.2 mg ICG and 50 mg PLGA were firstly dispersed in 1 mL acetone. The mixture was added to 40 mg BSA (bovine serum albumin) dispersed in 4 mL deionized water, and stirred overnight in the dark. PLGA-ICG-SiRB nanoparticles were obtained after purification of the suspensions by 8000–12,000 D dialysis bag.

2.2.3. Characterization of PLGA-ICG-SiRB

The PLGA-ICG-SiRB nanoparticles were characterized by transmission electron microscopy (TEM, Tecnai G2 20, FEI, USA). UV–vis absorption spectra were recorded using a UV–vis spectrometer (Lambda 35, Perkinelmer, USA). Fluorescence spectra were recorded with a RF-