Materials Science and Engineering C 78 (2017) 565–570

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

A biomimetic Au@BSA-DTA nanocomposites-based contrast agent for computed tomography imaging

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ARTICLE INFO

Article history:
Received 1 April 2017
Received in revised form 20 April 2017
Accepted 21 April 2017
Available online 22 April 2017

Keywords:
Gold nanoparticles
Biotemplate
Double-enhanced contrast agent
CT imaging

ABSTRACT

Early detection of cancer is increasingly important for being considered to increase the survival rate in the treatment process. The past decades years have witnessed the great progress in the biological detection application of gold nanoparticles. Herein, we reported a facile one-pot synthesis process to obtain gold nanoparticles (Au@BSA) with bovine serum albumin (BSA) as a biotemplate following with conjugation of diatrizoic acid (DTA) for a potential X-ray computed tomography (CT) imaging contrast agent (Au@BSA-DTA). The as-prepared biomimetic material was characterized systematically by several techniques. It was shown that the prepared biomaterial is colloid stable under the tested range of pH and temperature. The cell cytotoxicity assay, hemolytic assay and cell morphology observation showed that Au@BSA-DTA has good biocompatibility and hemocompatibility at a concentration of Au even up to 80 μg/mL. Besides, the biomimetic material Au@BSA-DTA with double radiodense elements of Au and iodine displayed much stronger CT imaging effect compared with the traditional small molecule contrast agents, which paves the potential clinical application in cancer early diagnosis.

1. Introduction

Tumor especially malignancy has remained a malignant disease that plagues human health, known as one of the killers of mankind, it may replace heart disease to be the chief cause of death in the near future [1]. At present, the clinical treatments of cancer mainly rely on radiotherapy, chemotherapy and other means. However, a class of side effects can be induced as a consequence of these techniques, which is obviously unfavorable to people's wellbeing [2,3]. Once cancers develop toward late, clinically existing treatments are almost impossible to heal. Therefore, it's particularly crucial to adopt early diagnoses and effective treatments without invasion for cancer. Much work so far has focused on biomedical imaging, which is widely used to explore the early biological changes, the level of metabolism, and genetic abnormalities because of its high specificity, sensitivity and image resolution [4–9]. Among them, X-ray computer tomography (CT) as an advanced non-invasive detection technology, is one of the most commonly used detection methods. Compared with other detection technologies, it owns many advantages such as strong penetration, high resolution, fast detection, intuitive test results, without contacting with the measured items and so on. Thus, the applications of CT have been intensively investigated since 20 century 70s [10–12].

For CT imaging, contrast agents are indispensable to enhance the imaging effect. Clinically used contrast agents are mainly iodine-containing reagents such as diatrizoate, iopromide, iodixanol and so on. But the following drawbacks of these iodinated small molecules should not be underestimated: (i) having short blood circulation time and imaging time; (ii) lacking of targeting and tissue specificity; (iii) accompanying with side effects and certain renal toxicity [13]. In order to solve these problems, looking for a kind of CT contrast agents with high radiation absorption coefficient, good water solubility and overcoming simultaneously the above shortcomings has drawn many researchers' extensive attention [14–17]. For example, dendrimers have been used to combine a water-insoluble iodinated agent (e.g., diatrizoate) to improve their properties including stability, biocompatibility and their blood circulation time [18–21]. And other macromolecules like liposomes and polymers have also been occupied to be served as carriers or capsules to surmount the inherent limitations of iodine-containing agents [22,23]. Besides, previous studies used biomacromolecule BSA as the biological template to conjugate some functional groups due to its good colloidal stability, water solubility and excellent biocompatibility as well as the superficial active sites to be conjugated with small molecule ligands [24–28].

Considerable research efforts have been devoted to explore metal elements such as bismuth, gold, platinum, which were contained into inorganic nanoparticles as CT contrast agents to achieve admirable imaging capability, improved detection and diagnose accuracy due to

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http://dx.doi.org/10.1016/j.msec.2017.04.127
0928-4931/

their relatively high atomic number and the greater attenuation coefficient for X-rays [27,29,30]. Notably, gold nanoparticles (AuNPs) with size < 100 nm have good physical and biological properties, unique surface plasmon resonance and good tissue compatibility. On the other hand, AuNPs are easy for surface functional modification and can be targeted easily [31–33]. Thus, the superiority of AuNPs makes them have extensive application especially in biomedical diagnose and therapy both in vitro and vivo [18,34–36].

In this work, we report a simple, green and low-cost method for the fabrication of Au@BSA, which was served as the nanocarrier for further functionalization of DTA. The fabricated novel CT contrast agent contained two kinds of active elements (gold and iodine) for enhanced X-ray absorption. First of all, we obtained the Au@BSA by reducing $AuCl_4^-$ with the reducer hydrazine hydrate and the stabilizer BSA, and the synthesis is a simple one-pot procedure under gentle experiment condition. Then, the conjugated Au@BSA-DTA was fabricated via amide coupling between the free amino group of Au@BSA and the carboxyl of DTA [18,37], followed by the characterization with the help of several techniques. Afterwards, the biocompatibility of the nanocomplex was proved by the assays of MTT and hemolysis, and its colloidal stability was also examined to be good as expected. X-ray absorption coefficient measurements confirmed that the two effective element-containing material has higher X-ray attenuation intensity in comparison with Au@BSA or clinical iodinated CT contrast agents. Generally, we constructed novel nanoparticles that may give new insights for the later CT imaging applications.

2. Materials and methods

2.1. Materials

$HAuCl_4$, bovine serum albumin (BSA, >99.8%, ~66.4 kDa), dimethylsulfoxide (DMSO), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), and *N*-hydroxysuccinimide (NHS) was purchased from Sigma-Aldrich (USA). Fetal bovine serum (FBS), 1640 cell culture medium were obtained from Xinbing Biotechnology Co., Ltd. (Shanghai, China). Diatrizoate (DTA), hydrazine hydrate ($N_2H_4 \cdot H_2O$) and other materials were all bought from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals and solvents were used as received. The water used in the whole experiments was deionized water purified by Millipore water purification system (18.2 MΩ, Milli-Q, Millipore). Regenerated cellulose dialysis membranes with a MWCO of 7000 were acquired from Fisher Scientific.

2.2. Methods

A Malvern Zetasizer Nano ZS90 instrument was used to perform dynamic light scattering (DLS) measurements with the sample particles dispersed in ultrapure water. UV–visible spectra were recorded on a Varian Cary-Eclipse 500 spectrometer. The morphology of the samples was characterized via a JEOL JEM-2100 transmission electron microscopy (TEM) operating at 200 kV. The sample dilute dispersion was dropped on a carbon-coated 200 mesh copper grid and air-dried for further imaging. FESEM was performed by a LEO1530 field-electron microscope (Germany) to collect the shape and EDS analysis of the nanoparticles. A Varian 720-ES (ICP-AES) was used to measure the metal contents.

2.3. Preparation of Au@BSA nanoparticles

A 1.25 g amount of lyophilized bovine serum albumin was dissolved in 50 mL deionized water and stirred to obtain the BSA solution. Then 20 mL of chloroauric acid solution (29.43 mM) was added rapidly under magnetic stirring. After continued stirring for 10 min, 100 μL hydrazine hydrate was added to the mixed solution. Subsequently, the mixture was left to react with constant stirring for 30 min, and the raw product was obtained.

2.4. Synthesis of Au@BSA-DTA

The pre-prepared Au@BSA was utilized to be the template to combine DTA moiety. Briefly, DTA (307 mg, 0.5 mmol) was suspended in 150 mL water with ultrasonic dispersion for 15 min. Following that, EDC (479 mg, 2.5 mmol) and NHS (143 mg, 1.25 mmol) was introduced to the dispersion solution and the mixture was stirred for 4 h. Then the synthesized Au@BSA was added dropwise to the above solution under gentle stirring for further 12 h. After that, a dialysis membrane (MWCO 7000) was employed to purify the resulting mixture against deionized water for 6 h. Finally, the Au@BSA-DTA nanoprobe was acquired after the procedure of lyophilizing.

2.5. Cytotoxicity assay in vitro

MCF-7 cells were used and obtained from American Type Culture collection and the cells grew regularly in 1640 cell culture medium with fetal bovine serum (10%) added. A humid atmosphere incubator with 37 °C and 5% CO_2 was necessary to incubate the cell lines.

A typical MTT assay in MCF-7 cell lines was investigated to evaluate the cell viability against Au@BSA-DTA. First of all, 8000 cells per well were seeded into 96-well plates with 5 replicates for each treatment group, and cultured by the cell medium 1640 supplemented with 10% FBS at 37 °C and 5% CO_2. After 12 h incubation, the complete culture medium containing varied concentrations of Au@BSA-DTA (0, 5, 10, 20, 30, 40 and 80 μg/mL) was added to each well after removing the original culture media and then grown at 37 °C under 5% CO_2 for another 24 h and 48 h, respectively. Next, the cells were incubated in 0.1 mL MTT (1 mg/mL) for additional 4 h after which discarded and 0.15 mL of DMSO per well was then added, followed by shaking for 15 min. At last, the 96 well plates were placed in a microplate reader (Thermo Multiskan Spectrum) to measure the absorbance value at 490 nm.

2.6. Hemolytic assay

Fresh human blood was used to verify the blood compatibility of the developed biomaterials, which was kindly provided by Jingzhou Central Hospital (Hubei, China). 1 mL fresh blood from human was placed in an Eppendorf tube to obtain the red blood cells (HRBCs) by a centrifuge at 2000 rpm for 10 min. The supernatant was discarded and washed with PBS, which was repeated 5 times. The purified HRBCs were diluted with 10 mL PBS. Then, 0.2 mL of HRBCs was mixed with 0.8 mL PBS and 0.8 mL water as a negative control and a positive control, respectively. The other 5 copies of diluted HRBCs suspension (0.2 mL) were added sequentially to the Au@BSA-DTA at different concentrations (10, 20, 30, 40, 80 μg/mL). All the samples were kept still for 2 h after a vortex, followed by centrifugation (12,000 rpm, 5 min). Afterward, A UV–visible spectrometer was utilized to record the absorbance of the supernatant (541 nm). We calculate the hemolysis percent of the mixtures using the absorbance at 541 nm as reported in literature [38].

2.7. X-ray attenuation measurement

For CT scanning, the samples (Au@BSA-DTA, Au@BSA, iopromide) with a series of Au or iodine concentrations (0, 5, 10, 15, 20, 40 mM) in 1-mL Eppendorf tubes were settled in a self-designed scanning holder to scan using a Brilliance 64-slice CT imaging system (Philips Healthcare, Andover, MA) under conditions of 120 kV, 200 mA, FOV = 25.0 cm and a slice thickness of 0.625 mm. Contrast enhancement was determined in Hounsfield units (HUs) for each sample mentioned above.