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Encapsulating tantalum oxide into polypyrrole nanoparticles for X-ray CT/photoacoustic bimodal imaging-guided photothermal ablation of cancer

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ABSTRACT

A nanotheranostic agent has been readily fabricated by encapsulating tantalum oxide (TaO_x) nanoparticles (NPs) into polypyrrole (PPy) NPs via a facile one-step chemical oxidation polymerization for bimodal imaging guided photothermal ablation of tumor. It was proved that the obtained composite nanoparticles (TaO_x@PPy NPs) with an average diameter around 45 nm could operate as an efficient bimodal contrast agent to simultaneously enhance X-ray CT and photoacoustic (PA) imaging greatly *in vivo*. Systemically administered TaO_x@PPy NPs could passively accumulate at the tumor site during the blood circulation, which was proved by both CT and PA imaging. In addition, the *in vivo* therapeutic examinations showed that TaO_x@PPy NPs exhibited significant photothermal cytotoxicity under near infrared laser irradiation. The tumor growth inhibition was evaluated to be 66.5% for intravenously injection and 100% for intratumoral injection, respectively. This versatile agent can be developed as a smart and promising nanoplatform that integrates multiple capabilities for both accurate diagnosing and precise locating of cancerous tissue, as well as effective photoablation of tumor.

1. Introduction

In recent decades, photothermal therapy (PTT) has attracted intensive interests as a minimally invasive cancer treatment which specifically burns tumor tissue using near infrared (NIR) laser irradiation in combination with photoabsorbers to avoid collateral damage to otherwise normal tissue. Nevertheless, for an effective and safe PTT treatment, the location and size of the tumors and the distribution of photothermal agents must be identified before PTT treatment and the therapeutic effectiveness has to be assessed after therapy. All these tasks could be carried out by integrating contrast-enhanced diagnostic imaging capability with PTT. Therefore, the imaging guided PTT was investigated as one of the most exciting strategies for cancer treatments and the relating photothermal agents with both therapeutic and imaging functions have received increased attention [1–4]. The combination of imaging detectability and therapeutic capacity in the same agent would avoid putting the additional stress on the body's blood clearance mechanisms caused by administration of multiple doses of agents.

Compared with the other medical imaging systems available, X-ray computed tomography (CT) takes more advantages in illustrating biological structures due to its high resolution and ease of forming 3D visual image for locating tissues of interest [5]. Yet, its inherently low sensitivity results in poor soft-tissue contrast. Recently, the idea of using multiple modalities in conjunction has gained in popularity. Thus, it is urgently needed to introduce a complementary imaging modality for accurate diagnosis using CT imaging. Compared with CT imaging, photoacoustic (PA) imaging is a neoteric and noninvasive imaging modality, which combines good spectral selectivity of laser light and high resolution of ultrasound detection. It can provide high spatial resolution even at several centimeters deep inside biological tissue to visualize tissue structure and functions [6,7], such as brain structural and functional imaging, breast cancer imaging, and tumor angiogenesis

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monitoring [8–11]. The signal-to-noise ratio (SNR) of PA can be further improved by carefully choosing the excitation laser wavelength within NIR region to minimize the attenuation of the light intensity [12]. In addition, exogenous contrast agents with high absorption in the NIR region have been developed to improve molecular sensitivity and expand the functionality of the PA [11,13]. Therefore, it is undoubted that the conjunction of CT and PA imaging would conduce to accurate locating of cancerous tissue for more precise guidance for PTT.

Due to the inherently low sensitivity and soft tissue contrast in CT imaging, it is often required to administrate a large amount of contrast agent for accurate diagnosis. Small iodinated compounds are widely used CT contrast agents in clinic but they are rapidly excreted via renal elimination, which may limit their applications for target specific imaging and angiography [14]. Gold nanoparticles (NPs) take more advantages to operate as X-ray CT contrast agents due to not only the high absorption coefficient of X-ray but also the outstanding biocompatibility. Nevertheless, the cost of using gold as X-ray contrast agent is a huge obstacle to their clinical utilization. Tantalum is much cheaper than gold while possessing a comparable X-ray attenuation coefficient (Ta, 4.3 and Au, 5.16 cm^2/kg at 100 eV). Recently, tantalum oxide (TaO_x) NPs have been demonstrated to be a strong candidate as a CT contrast agent due to its high X-ray attenuation coefficient and excellent biocompatibility. [15,16].

Currently, numerous materials with strong optical absorbance in the NIR region have been explored for PTT therapy, such as various gold nanostructures [1,17,18], CuS NPs [2,19–21], carbon nanomaterials [3,22,23], polypyrrole (PPy) NPs [24] and prussian blue (PB) NPs [25]. Because of the morphology-dependent NIR absorbing property, Au nanoshells [1,26,27], nanocages [17] and nanorods [28–30] have been extensively investigated as photothermal agents for photoablation of cancer [13,31]. Nevertheless, these NIR absorbing gold nanostructures have low photostability since their morphology and NIR absorption peak would diminish after a long period of laser irradiation due to the "melting effect" [32,33]. Among all the NIR absorbing nanomaterials, uniform PPy NPs are of particular importance as a promising photothermal agent for localized tumorous PTT due to the good biocompatibility, significant photothermal conversion efficiency, and remarkable photostability higher than gold nanostructures [34,35]. In addition, it has been demonstrated that PPy NPs show great potential for the use of a polymer-based nanoparticulate optical contrast agent for PA imaging [12].

Herein, TaO_x NPs were successfully encapsulated inside uniform PPy NPs via a facile one-step aqueous dispersion polymerization using polyvinyl alcohol (PVA) as a stabilizer for CT/PA bimodal imaging guided PTT (Fig. 1) [16,34,36,37]. PPy NPs function as a photothermal agent for photoablation of tumor and a contrast agent to enhance PAT imaging while TaO_x NPs operate as CT contrast agent. Both PAT and CT contrast behavior of the obtained TaO_x@PPy NPs were evaluated *in vitro* and *in vivo*. The photothermal cytotoxicity of the composite TaO_x@PPy NPs was investigated using both cancer cells and tumor-bearing nude mice.

2. Materials and methods

2.1. Materials

Pyrrole and Igepal CO-520 were purchased from Sigma–Aldrich and used without further purification. Cyclohexane was obtained from Tianjin Fengchuan Chemical Reagent Science and Technology Co., Ltd. Tantalum (V) ethoxide was purchased from J&K Chemical Ltd. (3-aminopropyl)triethoxysilane (APTES) was obtained from Aladdin Chemistry Co., Ltd. 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-dipheny- tetrazolium-romide (MTT) were obtained from Beijing Biotopped Science&Technology Co., Ltd. Deionized (DI) water was obtained by a Milli-Q Water Purification system.

2.2. Synthesis and modification of TaO_x NPs

The TaO_x NPs were prepared according to the reported method [16,37]. Briefly, microemulsion (ME) was prepared by adding 0.25 mL of NaOH aqueous solution (75 mM) to the oil phase composed of 2.3 g of Igepal CO-520, and 20 mL of cyclohexane. After 0.05 mL of tantalum (V) ethoxide (0.3 mmol) was added to the mixture at room temperature, a resulting mixture containing tantalum oxide nanoparticles (named as TaO_x-ME) was obtained within 5 min. To keep the stability of the TaO_x NPs, 145 μL of (APTES) was reacted with the obtained TaO_x-ME at room temperature for 24 h. The resulting nanoparticles were precipitated by adding a mixed solution of 1:1 (v/v) ether/*n*-hexane. After being washed several times with ether and deionized water, the final nanoparticles were dispersed in water and freeze-dried for 48 h.

2.3. Preparation of TaO_x@PPy NPs

TaO_x@PPy NPs were prepared via a chemical oxidation polymerization method [34,38]. Briefly, 1.50 g PVA was dissolved in 20 mL DI water with magnetically stirring at 60 °C for 20 min. After cooling to room temperature, $FeCl_3 \cdot 6H_2O$ (0.23 M, 1.2434 g) and TaO_x NPs (0.1354 g) was added, the system evolved from a clear state to a viscous khaki one. After 1 h to allow equilibration, 140 μL of pyrrole monomer was introduced into the aqueous PVA/TaO_x NPs/$FeCl_3$ solution and the polymerization proceeded at 5 °C while stirring. The mixture solution turned black within a few minutes. After stirring for 4 h, the polymerization completed. The resulting nanoparticles were separated from the free TaO_x NPs which were not encapsulated into PPy NPs through centrifugation (2000 rpm, 20 min) and washed several times with water, and the dispersion solution and blank PPy NPs were also removed by centrifugation (8000 rpm, 20 min) and washed several times with hot water to remove impurities. The obtained TaO_x@PPy NPs were re-dispersed with water by

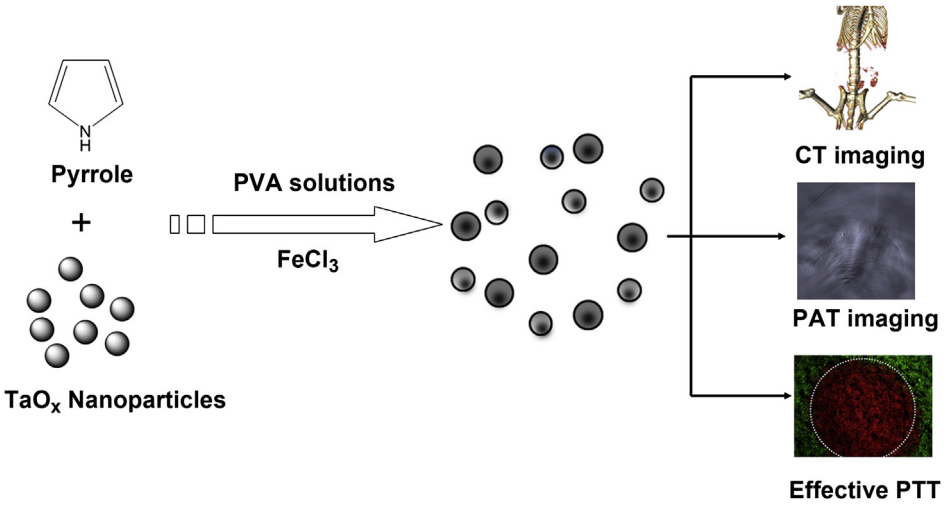

Fig. 1. Schematic illustration of the formation of TaO_x@PPy NPs for combined CT/PA imaging and PTT.