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Melanin-originated carbonaceous dots for triple negative breast cancer diagnosis by fluorescence and photoacoustic dual-mode imaging

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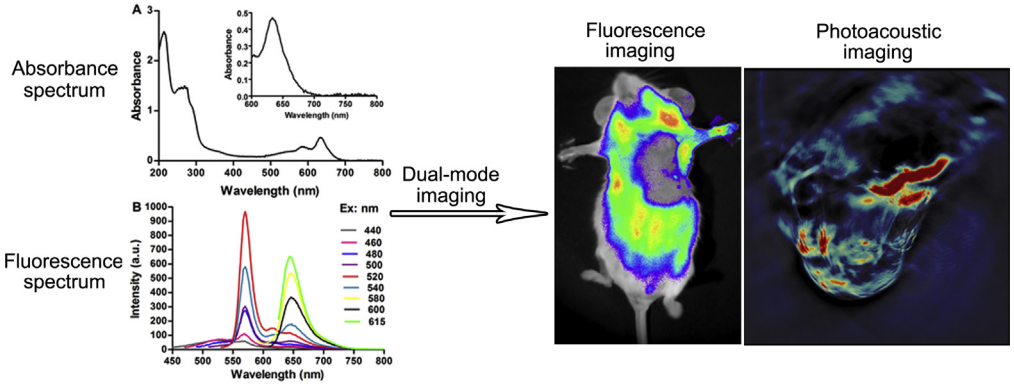
GRAPHICAL ABSTRACT



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ABSTRACT

Carbonaceous dots exhibit increasing applications in diagnosis and drug delivery due to excellent photostability and biocompatibility properties. However, relative short excitation and emission of melanin carbonaceous dots (MCDs) limit the applicability in fluorescence bioimaging. Furthermore, the generally poor spatial resolution of fluorescence imaging limits potential *in vivo* applications. Due to a variety of beneficial properties, in this study, MCDs were prepared exhibiting great potential in fluorescence and photoacoustic dual-mode bioimaging. The MCDs exhibited a long excitation peak at 615 nm and emission peak at 650 nm, further highlighting the applicability in fluorescence imaging, while the absorbance peak at 633 nm renders MCDs suitable for photoacoustic imaging. *In vivo*, the photoacoustic signal of MCDs was linearly correlated with the concentration of MCDs. Moreover, the MCDs were shown to be taken up into triple negative breast cancer cell line 4T1 in both a time- and concentration-dependent manner. *In vivo* fluorescence and photoacoustic imaging of subcutaneous 4T1 tumor demonstrated that MCDs could passively target triple negative breast cancer tissue by enhanced permeability and retention effects and may therefore be used for tumor dual-mode imaging. Furthermore, fluorescence distribution in tissue slices suggested that MCDs may distribute in 4T1 tumor with high efficacy. In conclusion, the MCDs studied offer potential application in fluorescence and photoacoustic dual-mode imaging.

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1. Introduction

Photoacoustic imaging combines the high contrast of fluorescence imaging with the high spatial resolution of ultrasound [1]. Since its introduction one decade ago, photoacoustic imaging has been one of the fastest growing technologies for bioimaging. Various kinds of contrast agents have been developed for photoacoustic imaging and disease diagnosis, including intrinsic chromophores, small molecular dyes, gold nanostructures and carbon nanotubes [2].

Carbonaceous dots (CDs) represent a new nanostructure type that has attracted increasing attention due to the high photostability, bright fluorescence, high biocompatibility and easily conducted structural modifications [3]. Generally, CDs are prepared using hydrothermal methods, microwave treatment or other techniques from various carbon sources [4]. In previous studies, we have developed several CDs exhibiting a long fluorescence excitation wavelength and a high quantum yield from silk, glutamic acid, glucose and graphene [5–8]. These CD types exhibited great potential in cancer imaging, while surface modifications with specific ligands, e.g. RGD and angiopep-2 [9–11], further improved the tumor imaging contrast. However, fluorescence imaging is generally limited by low spatial resolution. Therefore, introducing other imaging technologies, such as photoacoustic imaging, remains an important scientific goal to expand the applicability of CDs.

Until today, only several studies utilized CDs or fabricated CDs for photoacoustic imaging [12–15]. It is still not clear whether CDs could be used for fluorescence and photoacoustic dual-mode imaging or not. Therefore, in this study, we utilized our recently prepared melanin CDs (MCDs) [16] to evaluate potential applications in dual-mode imaging of triple negative breast cancer. Melanin represents a ubiquitous natural material found in skin, hair, and eyes. The pigment is an effective light absorber due to its high molar extinction coefficient, enabling the sensitive characterization of metastatic melanoma and melanoma cells [21]. MCDs prepared from melanin exhibit high biocompatibility and less toxicity, as well as excellent bioimaging abilities.

2. Materials and methods

2.1. Materials

Melanin (99%) was purchased from Keddia Reagent (Chengdu, China). 4′,6-diamidino-2-phenylindole (DAPI) was purchased from Beyotime (Haimen, China). Plastic cell culture dishes and plates were obtained from Wuxi NEST Biotechnology Co. Ltd (Wuxi, China). 4T1 cells were obtained from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Complete RPMI 1640 cell culture medium containing 10% of fetal bovine serum (FBS), 100 U mL^{-1} of penicillin G and 100 U mL^{-1} of streptomycin sulfate was obtained from Life Technologies (Grand Island, USA). Female Balb/c mice (20 ± 2 g) were purchased from Dashuo Biotechnology Co. Ltd, (Chengdu, China) and were maintained under standard housing conditions. All animal experiments were carried out in accordance with guidelines evaluated and approved by the ethics committee of Sichuan University, China.

2.2. Preparation and characterization of MCDs

MCDs were prepared directly using a hydrothermal method as described previously in the literature [16]. Briefly, 1.0 g of water-soluble melanin was dissolved in 6 mL of deionized water and the reaction mixture was heated at 220 °C for 48 h. After reaction completion and cooling to room temperature, the solution was filtered (pore size 0.45 μm). The morphology of the MCDs was captured by transmission electronic microscopy (TEM) (Tecnai G2 F20 S-TWIN, FEI, USA). A ultraviolet–visible (UV–vis) spectrum was recorded in water using a Varian cary 100 conc UV–vis spectrophotometer (Varian, USA). A fluorescence spectrum was recorded using a Shimadzu RF-5301PC spectrofluorophotometer (Shimadzu, Japan).

2.3. Evaluation of the photoacoustic properties of MCDs

A series of MCDs concentrations were used for tissue-mimicking phantom imaging. The photoacoustic signal was recorded using multi-spectral photoacoustic tomography (MSOT in Vision 128, iTheramedical, Germany).

Phantom preparation and imaging procedures were as follows [23]: Agar (Fluka-05039) providing phantom rigidity and intralipid (Sigma-I141) producing tissue-like scattering were used to prepare a cylindrical phantom with a diameter of 2 cm. Specifically, per 100 mL final volume, 1.3 g of agar and 5 mL of 20% intralipid were added to 94 mL of deionized water and the mixture was heated until boiling. Then, the mixture was cooled to about 50 °C and was poured into a 20-cc syringe. A plastic straw was cut to 3–4 cm in length and each side was sealed with glue. Two straws were simultaneously and partially submerged in the center of the phantom. The straws were stabilized until the phantom solidified. Subsequently, two different straws containing MCDs and the control agent were allowed to replace the air-filled straws. Finally, the phantom was imaged and the straws were replaced in between scans.

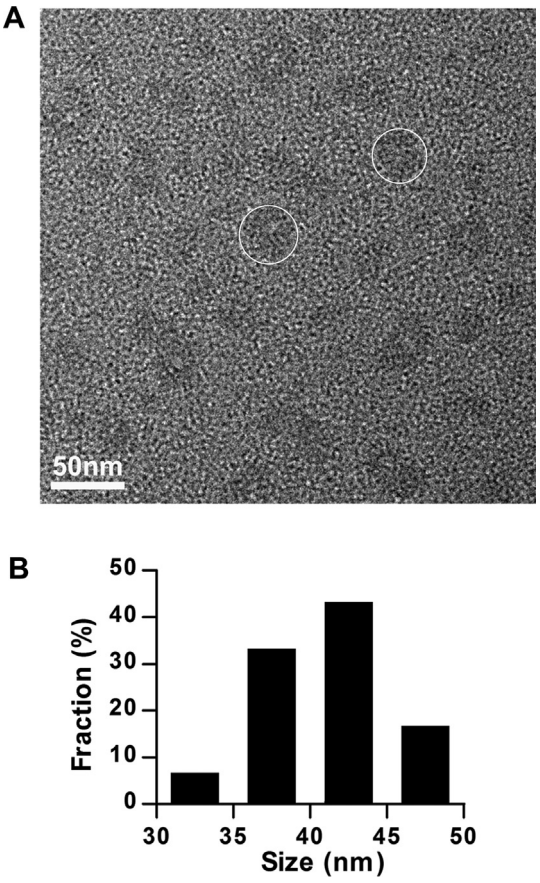

Fig. 1. (A) TEM image of MCDs, the circles represent MCDs and the bar represents 50 nm. (B) Size distribution of MCDs.