Fluorescent C-dot Nanocomposites as Efficient Photothermal Agents and Multi-modal Imaging Tracers

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Supporting Information

Experimental Section

1. Materials

Methylene blue (MB) was purchased from J&K Co. Ltd. without further purification. Soybean milk was obtained from Yonho Food Co., Ltd. Phosphate buffer saline (PBS), paraformaldehyde, penicillin and streptomycin were purchased from Aladdin. Dulbecco modified Eagle medium (DMEM), L15 medium and fatal bovine serum (FBS) were obtained from Sigma-Aldrich.

2. Synthesis of C-dots (CDs) and C-dot nanocomposites (MB-CDs)

The synthetic protocol of C-dots without MB-embedding (CDs) was slightly modified as previously reported.[S1,S2] Briefly, 2.5 mL fresh soybean milk in a reactor vial was put into a microwave synthesizer (Initiator 4.1.2, Biotage, Sweden) and heated at 160 °C for 5 h. After cooling down to room temperature, the solution was centrifuged (3×30 min, 10,000 rpm, 25 °C) to remove impurities, and the supernatant was dialyzed with a dialysis bag (MWCO=1,000 kDa, MYM Technologies Ltd., India) in Milli-Q pure water for 2 h before lyophilization.

For the preparation of C-dot nanocomposites (MB-CDs), MB (50 mg, 0.16 mmol) was dissolved in 100 mL Milli-Q pure water as a stock solution. Then 2.5 mL of MB stock solution and 2.5 mL fresh soybean milk were mixed together and put into the microwave synthesizer at 160 °C for 5 h. After cooling down to room temperature, the solution was centrifuged (3×30 min, 10,000 rpm, 25 °C) to remove impurities, and the supernatant was dialyzed with a dialysis bag (MWCO=1,000 kDa) in Milli-Q pure water for 2 h before lyopholization.

3. Characterization

The photoluminescence (PL) spectra were obtained on a spectrofluorometer (FluoroMax-4C-L, Horiba Scientific Instrument Inc., USA) with progressively longer excitation wavelength from 340 nm to 440 nm in 20 nm increments. The UV-Vis absorption spectra were acquired on a spectrophotometer (Lambda 25, PerkinElmer, USA). The FT-IR spectroscopy (Bio-Rad FTS-6000, Digilab Division, USA) was applied to measure the characteristic peaks and to identify the chemical structures of MB, CDs and MB-CDs. Transmission electron microscopy (Tecnai G2 20, FEI, USA) was used to visualize the morphology of MB-CDs and randomly select 100 particles to calculate the size (Mean±SD). The zeta potential was measured by a Zetasizer (Nano ZS, Malvern Instrument Inc., Worcestershire, UK), and triplicate measurements were performed to afford the zeta data (Mean±SD). The confocal laser scanning microscope (A1 PLUS, Nikan, Japan) was utilized to visualize the endocytosis of MB-CDs and passive accumulation into MDA-MB231 in a dual-channel imaging manner at the excitation wavelength of 405 nm and 640 nm, respectively.

4. Photothermal imaging and temperature increase analysis

1 mL of free MB (125 μM), CDs, MB-CDs ([MB]=125 μM) and Milli-Q pure water were added into the transparent cuvettes, respectively. A pulsed laser (WG 1533D3, Beijing Energy Optoelectronics Technology Co., China) was fixed above the samples, and irradiation at 808 nm at different energy density (1-3 W/cm^2) and varied distances (1-5 cm) were conducted for 5 min. The real-time thermal images and temperature changes were recorded at a 30s-interval with an infrared thermal imaging camera (S6, IRS Systems Inc, China). The temperature increase curves were generated in correlation to different energy density and varied distances.

For the phantom photothermal imaging, 500 μL of MB-CDs ([MB]=156 μM) were injected into a piece of fresh pork (2×2×0.5 cm) as simulated tissue in comparison to the injection of 500 μL Milli-Q pure water as a control, and then laser irradiation (808 nm, 3 W/cm^2, 10 min) were conducted. The real-time thermal images and temperature changes were recorded at a 1min-interval with the infrared thermal imaging camera.

5. Cell cultures

Human breast cancer cells MDA-MB231 was purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. MDA-MB231 was cultured with L15 media supplemented with 5% FBS, 50 IU/mL penicillin and 50 μg/mL streptomycin. All of the cells were cultured at 37 ℃ in a humidified environment of 5% CO_2.

6. In vitro cellular studies

MDA-MB231 cells were seeded in 60 mm cell tissue-culture plate at a density of 3×10^5 per well. After incubation for 24 h, the plate was washed with PBS to remove the non-adherent cells and replaced with fresh medium containing MB-CDs ([MB]=100 μL, 10.8 μM). After co-incubation for 30 min, the cells was fixed with 4% paraformaldehyde followed by confocal laser scanning microscopy for dual-channel tracking at the wavelength of 405 nm and 640 nm, respectively.

7. Photoacoustic imaging of ocular blood vessels with MB-CDs

Female nude mouse was obtained from Shanghai SLAC Laboratory Animal Co. Ltd. All animal experimental procedures have been approved by the Administrative Panel of Wenzhou Institute of Biomaterials and Engineering, Wenzhou Medical University, and these methods were carried out in accordance with the animal experimental guidelines of Wenzhou Medical University. 200 μL of freshly prepared C-dot nanocomposites MB-CDs ([MB]=125 μM) was intravenously injected via the tail vein. The mouse was fully anesthetized with isoflurane (1.5% mixed with oxygen, RWD Life Science, China). An in vivo photoacoustic small-animal imaging system (LAZR, Visualsonics, Canada) with a transducer (LZ400, Visualsonics, Canada) frequency of 30 MHz at a laser wavelength of 680 nm was conducted to capture merged PA and ultrasound images within 30 min post-injection. A defined ocular area was selected as the region of interest (ROI) for PA imaging of ocular blood vessels.

References

S1. X. Yang, X. Yang, Z. Li, S. Li, Y. Han, Y. Chen, X. Bu, C. Su, H. Xu, Y. Jiang, Q. Lin, *J. Colloid Interface Sci.*, 2015, **456**, 1–6.

S2. J. Duan, J. Yu, S. Feng and L. Su, *Talanta*, 2016, **153**, 332-339.

Figures

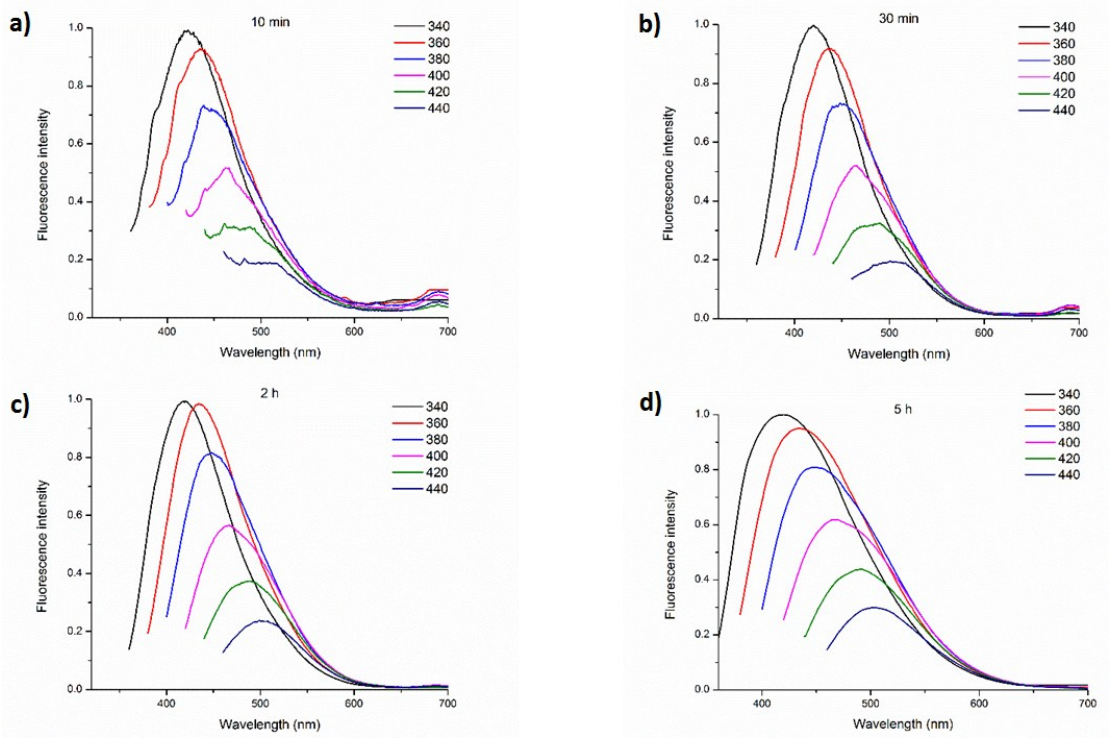

Fig. S1 Photoluminescence measurements at the excitation wavelength of 340~440 nm to demonstrate the optimal microwaving time (5 h) at 160 ℃ for the synthesis of MB-CDs.

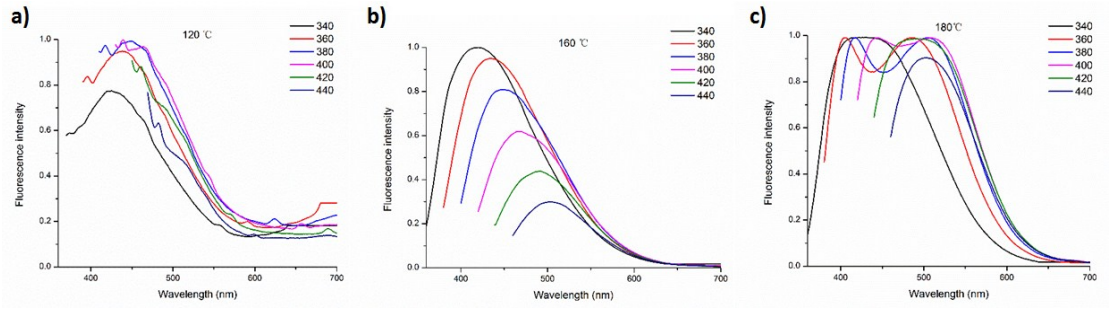

Fig. S2 Photoluminescence measurements to demonstrate the optimal temperature (160 ℃) for 5-hour microwaving for the synthesis of MB-CDs.

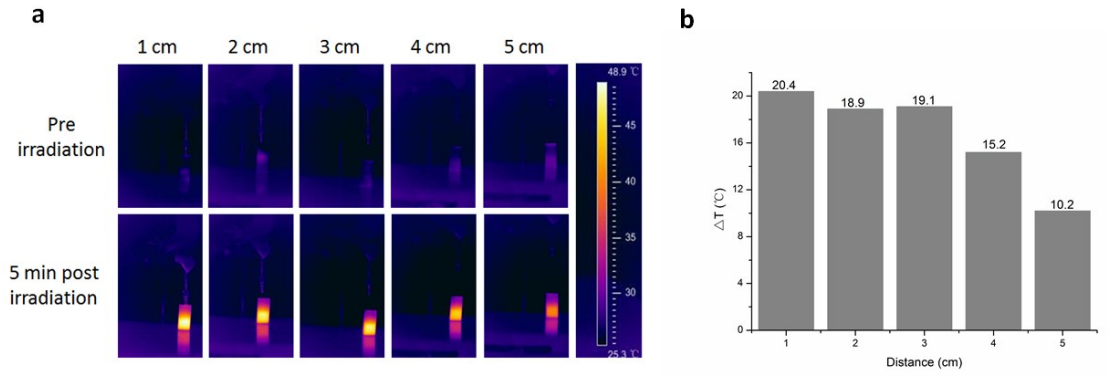

Fig. S3 Photothermal imaging and relationship between temperature increases and laser irradiation distance. a) Photothermal images with different distances (1, 2, 3, 4 and 5 cm) from laser point to MB-CDs solution. b) Variation of temperature increases while distance from laser point to MB-CDs solution changes.