Facile Synthesis of Hydrophilic Multi-color and Upconversion Photoluminescent Mesoporous Carbon Nanoparticles for Bioapplications

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Experimental Section

Chemicals.

All chemicals were used as received without further purification. Citric acid monohydrate (Sinopharm Chemical Reagent Co.), Ethanol (Shanghai Lingfeng Chemicals Co.), 1-octadecene (Sigma–Aldrich, technical grade, 90%), Dhydrogen dioxide(Sigma–Aldrich, technical grade, 30%). Milli-Q water (0.66MΩ·cm @27.2 °C) was used in the entire experiments.

Characterization.

Transmission electron microscopy (TEM) images were recorded on JEM-2100F electron microscope at 200 kV by dropping diluted sample on carbon-coated copper grid. Particle size distribution measurements by dynamic light scattering (DLS) were carried out at 25 °C on Zetasizer instrument (PSS Nicomp TM 380ZLS,USA). UV/Vis spectra were collected at room temperature on a UV-3101PC Shimadzu spectrophotometer. Fluorescence spectra were acquired on a RF-5301 Shimadzu spectroscope with a 500 W continuous-wave xenon lamp with corresponding optical filter (λ = 640±15, 670±15, 700±15, 740±15, 800±15, 880±15nm) purchased from Accute Optical Technology Co. Raman spectroscopy spectra was recorded on a DXR Raman Microscope, with an excitation length of 532 nm. FTIR spectra was measured by a Nicolet Magna 500 FTIR spectrophotometer. Nitrogen physiosorption isotherms of dried sample were obtained with a Micromeritics Tristar 3000 physisorption instrument. Surface areas and pore size distributions were determined respectively according to the Brunauer-Emmett-Teller (BET) method and the Barrett-Joyner-Halenda (BJH) method. Confocal microscopy images were obtained on a FV 1000 Olympus confocal laser scanning microscope. Fluorescence quantum yields were measured on a FluoroMax-4 spectrophotometer (Horiba),which is equipped with an
integrating sphere Quanta-ϕ. The optical filter (λ = 340-380 nm) were used to excite the samples while the optical filter (λ = 420-480 nm) detected.

**Preparation of mesoporous carbon nanoparticles.**
The mesoporous carbon nanoparticles were prepared by a simple precursor carbonization route in hot organic solvent (denoted as PC-in-HS) approach. Briefly, 15mL 1-octadecane was placed into a 50mL three-necked flask and degassed with nitrogen. Upon reaching 240 °C, 2 mL citric acid solution (0.5g citric acid in 2 mL ethanol) was added quickly to the solution with vigorous stirring and kept under the temperature for 15 min. The products were extracted with water and further purified by placing into dihydrogen dioxide solution under 80 °C for 1 hour. The final products were dialyzed against water for 24 hours and then dried in the oven.

**Cell Culture and MTT Assay**.
Routinely Hela cells were cultured to test the cellular cytotoxicity of the mesoporous carbon nanoparticles. Hela cells were initially plated in 96-well plates at a density of approximately 1×10^4 cells per well and were cultured in DMEM medium with 10% fetal bovine serum (FBS) in a 5% of CO₂ humid incubator for 24 h at 37.8 °C. Then the mesoporous carbon nanoparticles were added into the medium at increasing concentrations of 12.5, 25, 50, 100 µg/mL and the cells were incubated for 24 h at 37.8 °C. The cells cultured with pure culture medium were set as controls. Cell viability was assessed by a standard MTT assay (Sigma, St. Louis MO).

**In vitro Confocal Luminescence Imaging.**
Hela cells were seeded and cultured in DMEM medium supplemented with 10% fetal bovine serum at 37.8 °C in a 5% of CO₂ for 24 h. A concentration of 0.05 mg/mL of the mesoporous carbon nanoparticles was incubated with Hela cells at 37.8 °C for 12 h, and the cell sample was washed with phosphate buffer solution (PBS, pH = 7.4) for three times. Confocal luminescence imaging of Hela cells was aquired on a Olympus FV1000 laser-scanning confocal microscope. More experiment details: The excited light source is argon ion laser equipped with 340, 488, 637nm laser and the scan speed is 4.0us/pixel. All the image is taken under OLYMPUS PLAPON 60X at 1.42 numerical aperture and the scanning pinhole size is 140 µm.

**Drug loading and release.**
The MCNs-DOX were obtained by immersing 5.0 mg of MCNs in a 10 mg of DOX aqueous solution (2 mg/mL) at room temperature under light-sealed conditions and the mixture was magnetically stirred for 24 h. To evaluate the DOX loading efficiency, the supernatant DOX solutions were collected by centrifugation. The concentration of unloaded DOX was measured by UV-vis measurement at 485 nm, via refereeing to a calibrated linear curve of standard DOX solutions in PBS of concentrations ranging from 0 to 0.5 mg/mL. The drug loading capacity could then be easily obtained from the remained DOX concentration of the supernatant. DOX drug loading amount was measured and calculated using the below equation:
Loading amount (\%) = 100 \times \frac{\text{total DOX used-DOX in supernatant}}{\text{total MCNs used + total DOX used-DOX in supernatant}}.

4 mg of DOX-loaded MCNs was put into a dialysis bag (cut off molecular weight 8000 g/mol), then the sealed dialysis bag was put into a tube containing 20 mL PBS (pH 7.4). The tubes were shaken at 140 rpm at 37 °C under light-sealed condition. At certain time intervals, 3.0 mL solution was taken out to measure the released DOX concentration by UV-vis absorption, then put back to the tube.

**Assessment of cytotoxicity in Hela cells treated with DOX-loaded MCNs and free DOX**

Hela cells were seeded in 96-well plate at a density of \(1 \times 10^4\) cells in DMEM medium with 10% fetal bovine serum (FBS) in a 5% of CO\(_2\) humid incubator for 24 h at 37.8 °C. Then the DOX-loaded MCNs and free DOX were added to the medium at increasing concentrations of 0.1, 1, 5, 10 \(\mu\)g/mL and the cells were incubated for 24 h and 48 h at 37.8 °C. Cell vitality was assessed by a standard MTT assay.

![Figure S1. UV/Vis absorption spectra of the synthesized MCNs.](image)
Figure S2. BET nitrogen adsorption/desorption isotherms and BJH pore size distribution (inset) of the MCNs.
**Figure S3.** FTIR spectra of mesoporous carbon nanoparticles. Peaks at around 3445 cm\(^{-1}\) were attributed to the OH stretching modes, while those at 2920, 2850 and 1750 cm\(^{-1}\) indicate the presence of O=C-H. Bands at 1603, 1513 and 1458 cm\(^{-1}\) correspond to the C=C stretching mode of the polycyclic aromatic hydrocarbons, which also shows C-H bending vibrations at 875 and 802 cm\(^{-1}\). The peaks at around 1253 and 1011 cm\(^{-1}\) indicate the C-O stretching mode of aromatic hydrocarbons and regular carbon atoms.
Figure S4. XPS spectra of mesoporous carbon nanoparticles. A) Survey spectrum. B) C\textsubscript{1s} spectrum. C) O\textsubscript{1s} spectrum. The survey spectrum of the MCNs shows two typical peaks of C\textsubscript{1s} and O\textsubscript{1s}. The spectrum of C\textsubscript{1s} can be deconvoluted into three single peaks which correspond to C-C(284.8 eV), C-O(286 eV) and C=O(289.2 eV) functional groups, which have a good consistence with the results of FTIR. The
Figure S5. Raman spectrum of the synthesized MCNs, which displays two broad peaks at around 1330 and 1600 cm\(^{-1}\), owning to the D-band (sp\(^3\)) and G-band (sp\(^2\)) of carbon, respectively. The coexistence of both bands suggests that the mesoporous carbon nanosphere are amorphous.
Figure S6. Viabilities (%) of Hela cells incubated against MCNs for 24 h at 37 °C at varied MCN concentrations of 12.5, 25, 50, 100 and 200 μg/mL.

Figure S7. Schematic illustration of the PL mechanisms in MCNs showing that MCNs luminescence primarily originates from electron transitions between energy levels. A)
The mechanism of upconversion luminescence; B) The mechanism of downconversion luminescence.