Supplementary material

Mahonia-Derived Carbon Dots for Dual-Ion Ratiometric Sensing and Cellular Bioimaging

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1. Materials and methods

1.1 Materials

All chemicals used in this study were of analytical grade. MC was obtained from Nanning, (Guangxi, China). *Lycium barbarum* L. from Zhongning (Ningxia, China). *Dioscorea opposita* Thunb. from Wenxian (Henan, China). *Lonicera japonica* Thunb. from Xinxiang (Henan, China); and *Crataegus pinnatifida* Bge. from Shantou (Guangdong, China). All TCMs were purchased from certified pharmacies and authenticated by the Shenzhen Institute for Drug Control (Guangdong, China). m-PD, nitric acid (HNO_3, 99.7%, m/m), hydrochloric acid (HCl), hydrogen peroxide (H_2O_2, 30%, v/v), silver nitrate (99%, m/m), cerium sulfate (97%, m/m), cobalt (II) nitrate hexahydrate (99.99%, m/m), copper (II) nitrate trihydrate (99%, m/m), sodium chromate (98%, m/m), chromium (III) chloride hexahydrate (99%, m/m), and cadmium nitrate tetrahydrate (99%, m/m) were obtained from Aladdin (Shanghai, China). Iron (III) chloride (97%, m/m) was purchased from Sigma (Germany). Ferrous sulfate (98%, m/m), sodium chloride (99%, m/m), potassium chloride (99.99%, m/m), mercury (II) nitrate monohydrate (99.7%, m/m), calcium chloride (99%, m/m), magnesium chloride (99%, m/m), tin (II) chloride dihydrate (98%, m/m), zinc chloride (98%, m/m), nickel chloride (99%, m/m), quinine sulfate (98%, m/m), and lead nitrate (99.0%, m/m) were supplied by Macklin (Shanghai, China). Distilled water was used throughout all experiments. For cell-based assays, RPMI-1640 medium and the penicillin-streptomycin mixture were purchased from Gibco, while fetal bovine serum (FBS) was obtained from Biosharp. The Cell Counting Kit-8 (CCK-8) was sourced from Dojindo Laboratories (Shanghai), and 96-well plates were purchased from Costar (USA). A549, HCC827 and NHBE cell line are primarily sourced and distributed by the China Center for Type Culture Collection (CCTCC) in Wuhan, China.

1.2 Characterization

The optical properties of the MC-CDs were analyzed using a suite of spectroscopic techniques. UV-Vis absorption spectra were recorded on a UV-2600 spectrophotometer (Shimadzu, Japan), and fluorescence emission profiles were obtained using an F-7100 fluorescence spectrophotometer (Hitachi, Japan). Time-resolved photoluminescence measurements were performed with an M072 multifunctional transient fluorescence spectrometer (Edinburgh Instruments, UK). MC-CDs were synthesized in a 50 mL Teflon-lined stainless-steel autoclave, and the hydrothermally produced materials were subsequently freeze-dried using an LGJ-25D vacuum freeze dryer (Beijing Sihuan Qihang Technology Co., China). Electrochemical characterization was carried out on a CHI 660E workstation using a conventional three-electrode configuration. The microstructure and morphology of the samples were examined by high resolution transmission electron microscopy (HR-TEM, JEOL JEM-2100). Fourier-transform infrared spectra (FT-IR) were collected on an IRTracer-100 spectrometer (Shimadzu, Japan), while powder X-ray diffraction patterns were acquired using a Bruker D8 Advance diffractometer. Elemental composition and chemical states were investigated through XPS using a Thermo Scientific Escalab platform. Particle size distribution and zeta potential were determined with a Malvern Zetasizer Nano-ZS. Quantitative analysis of metal ion content was performed using an Agilent 7800 ICP-MS system (Omaha, Nebraska, USA). Cellular imaging studies were conducted on a Nikon Ti2-E fluorescence microscope (Japan), and flow cytometric analysis was carried out using a CytoFlex flow cytometer.

1.3 Synthesis of MC-CDs

Briefly, 1 g of MC powder (passed through a No. 3 sieve) and 0.4 g of m-PD were accurately weighed. The MC powder was dispersed in 30 mL of a diluted HCl solution (HCl : water = 1 : 99, v/v) and soaked for 30 min, followed by ultrasonic treatment (500 W, 40 kHz) for 45 min[1]. After settling, a portion of the supernatant was collected to dissolve m-PD, assisted by mild sonication to accelerate dissolution.

When m-PD was fully dissolved and no further gas bubbles were observed, the solution was combined with the original extract and sonicated again for 5 min to ensure homogeneity. The resulting mixture was centrifuged at 12,000 rpm for 15 min, and the supernatant was transferred into a Teflon-lined autoclave for hydrothermal treatment at 160-200°C for 4-8 h. After cooling down, the reaction mixture was filtered through a 0.22 μm membrane to remove residual particulates, and the filtrate was collected. Photographs and fluorescence responses of the samples were recorded under different testing conditions to monitor any color changes. The final products were stored at low temperature for subsequent use.

1.4 Determination of QY

$$QY_{MC\text{-}CDs} = QY_{ref} \times \frac{FL_{MC\text{-}CDs}}{FL_{ref}} \times \frac{A_{ref}}{A_{MC\text{-}CDs}} \times \frac{\eta_{MC\text{-}CDs}^{2}}{\eta_{ref}^{2}} \quad (1)$$

The fluorescence QY of the MC-CDs was evaluated using a standard comparative method based on equation (1) [2], with quinine sulfate dissolved in 0.1 M H_2SO_4 serving as the reference (ref). Quinine sulfate was selected because its emission range and excitation wavelength better matched the blue emission region of MC-CDs. In the calculation, fluorescence denotes the integrated fluorescence emission intensity obtained under 280 nm excitation, and A represents the corresponding absorbance measured from the UV-Vis spectrum. To minimize inner filter effects, the absorbance values of both quinine sulfate and the MC-CDs were maintained below 0.05. The term η refers to the refractive index of the solvent. when identical solvents are used for the sample and ref, the refractive index correction factor is taken as 1.

1.5 Fluorescence and ICP-MS Analysis of Ag^+ and Cr^{6+}

Fluorescence measurements for Ag^+ and Cr^{6+} detection were carried out under identical experimental conditions. MC-CDs were first dissolved in distilled water at 25°C, and the fluorescence spectrometer was configured with a PMT voltage of 500 V, slit widths of 3 nm, and a scanning speed of 1200 nm/min. Excitation wavelengths were screened within the 230-320 nm range to determine the optimal

excitation/emission pair. Subsequently, varying concentrations of Ag^{+} (0-3 μM) or Cr^{6+} (0-40 μM) were introduced into the MC-CDs working solution (0.05 mg/mL), and the corresponding fluorescence responses were recorded to construct calibration curves. Each measurement was performed in triplicate to ensure reproducibility and reliability. Selectivity was assessed through competitive assays by individually adding various metal ions (Hg^{2+}, Ca^{2+}, Cd^{2+}, Cu^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}, Na^{+}, K^{+}, Ce^{4+}, Al^{3+}, Cr^{3+}, Mg^{2+}, Zn^{2+}, Ni^{2+}, Sn^{4+}, Pb^{2+}) to the MC-CDs solution (0.025 mg/mL). Fluorescence intensities were recorded 1 min after ion addition and compared with the blank control to evaluate potential interference and confirm the selectivity and consistency of the sensing system. To further validate the analytical accuracy of the fluorescence method, results were compared with those obtained using ICP-MS. ICP-MS parameters were set as follows: pump speed, 20 rpm; nebulizer gas flow, 1.00 L/min; auxiliary gas flow, 1.00 L/min; sample rinse time, 40 s; and RF power, 1500 W.

1.6 Pretreatment of TCMs Samples

Following previously reported procedures [3], 0.3 g each of four above-mentioned TCMs were accurately weighed and placed into separate digestion vessels. Each sample was pre-digested with 6 mL of HNO_3, followed by the addition of 2 mL of H_2O_2 to enhance oxidation. Microwave-assisted digestion was then performed as follows: the temperature was increased to 120°C over 15 min and maintained for another 15 min, then further raised to 190°C within 10 min and held for 30 min to ensure complete decomposition of the samples. After digestion, the vessels were allowed to cool naturally to room temperature. The resulting digests were diluted with distilled water and brought to a final volume of 50 mL for subsequent fluorescence measurements and ICP-MS analysis.

1.7 Cytotoxicity Assessment and Cellular Imaging of MC-CDs

A549 and HCC827 cells were seeded in 6-well plates at a density of 3×10^3 cells per well and cultured in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in a 5% CO_2 atmosphere for 24 h. After incubation,

MC-CDs were added to each well at final concentrations of 0, 100, and 150 μg/mL, followed by an additional 24 h of culture. Cell viability was then evaluated using the CCK-8 assay according to the manufacturer's instructions to determine the potential cytotoxicity of MC-CDs. For cellular imaging, A549 cells and normal cell lines (NHBE) were seeded in 6-well plates and cultured to approximately 50% confluence, then maintained under the same conditions until stable growth was achieved. Cells were subsequently treated with 200 μL of MC-CDs at various concentrations (0, 50, 100, 150 μg/mL) for 24 h. After incubation, cells were washed three times with PBS to remove any non-internalized MC-CDs. Fluorescence images were acquired using 340 nm excitation to visualize the intracellular distribution of MC-CDs.

1.8 Flow Cytometry Analysis

Cells were seeded in 12-well plates at a density of 2×10^5 cells per well and incubated for 24 h before treatment. After stimulation, the cells were harvested and stained in the dark for 15 min using an Annexin V/FITC apoptosis detection kit (Cat. No. P-CA-201, Pricella, Wuhan Pricella Biotechnology Co., China). Subsequent procedures were carried out following the manufacturer's instructions, and the stained samples were analyzed within 1 h using a BD FACSCelesta flow cytometer (BD Biosciences, New Jersey, USA).

2. Results and discussions.

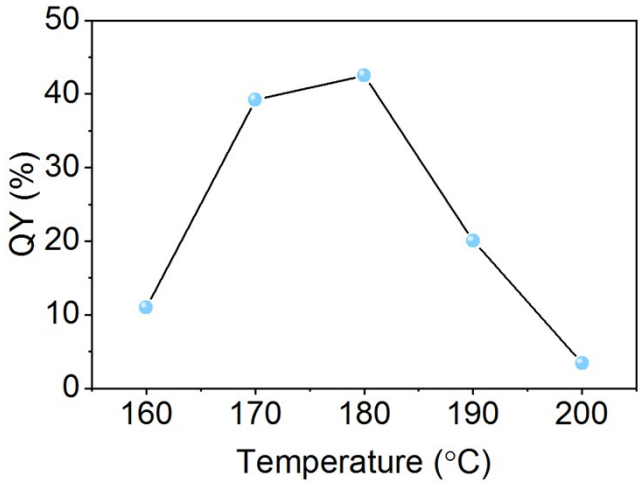

Fig. S1. Intensity of MC-CDs prepared under different temperature.

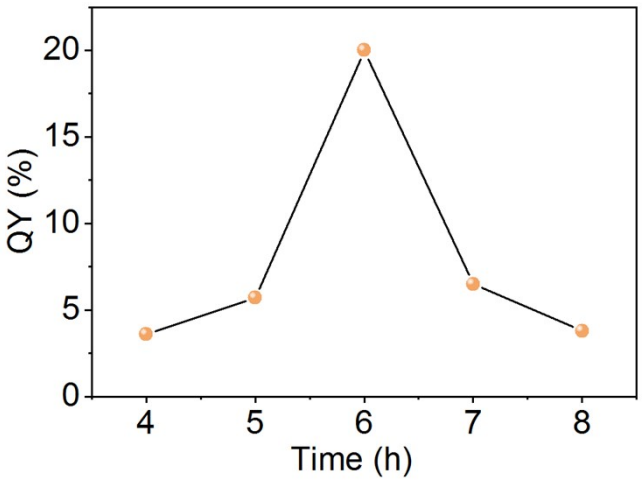

Fig. S2. Intensity of MC-CDs prepared under different reaction time.

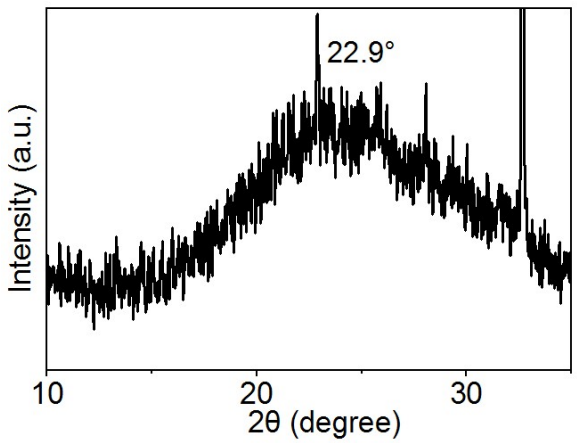

Fig. S3. XRD spectrum of MC-CDs.

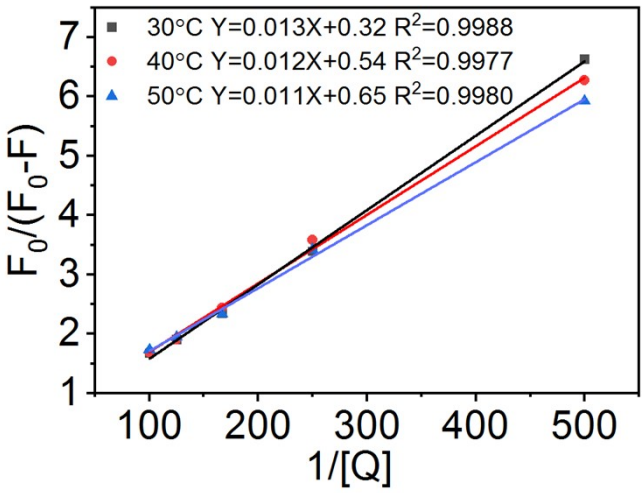

Fig. S4. Modified Stern-Volmer quenching curves of Cr^{6+} solution to MC-CDs at different temperatures.

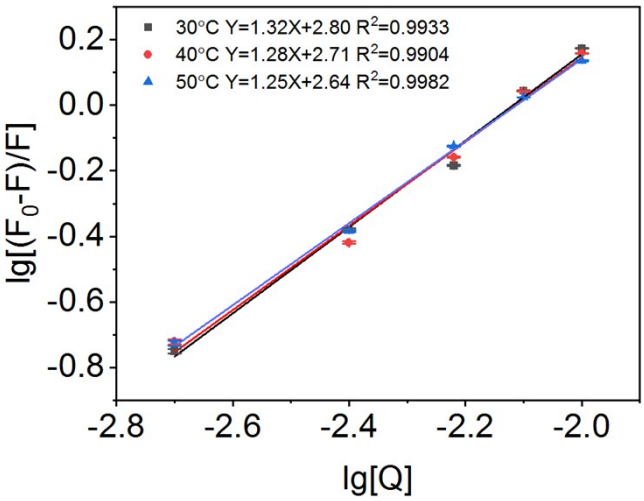

Fig. S5. Relationship curves between lg[(F_0-F)/F] and lg[Q] in the interaction of MC-CDs and Cr^{6+}.

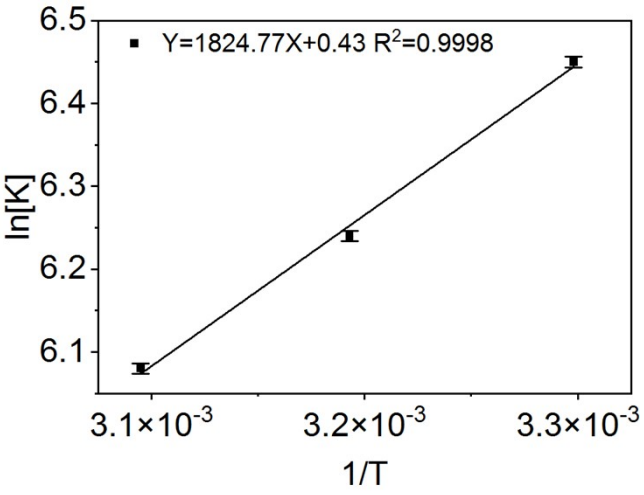

Fig. S6. Vant Hoff diagram of MC-CDs and Cr^{6+}

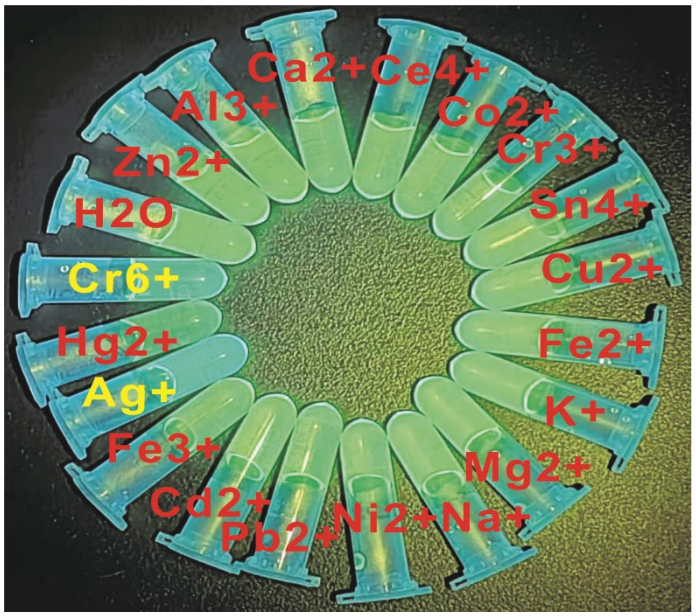

Fig. S7. Photography of MC-CDs solution after addition of different ions.

Table S1. Thermodynamic parameters of interaction between MC-CDs and Ag^{+} or Cr^{6+} at different temperatures

T/°C	K/L/moL	ΔH/kJ/moL	ΔG/kJ/moL	ΔS/J/mol·K	R^2	n
30	816.6	-36.24	-89.08	174.3	0.9998	0.9938
40	213.9	-36.24	-90.83	174.3	0.9998	0.9475
50	97.54	-36.24	-92.57	174.3	0.9998	0.9154

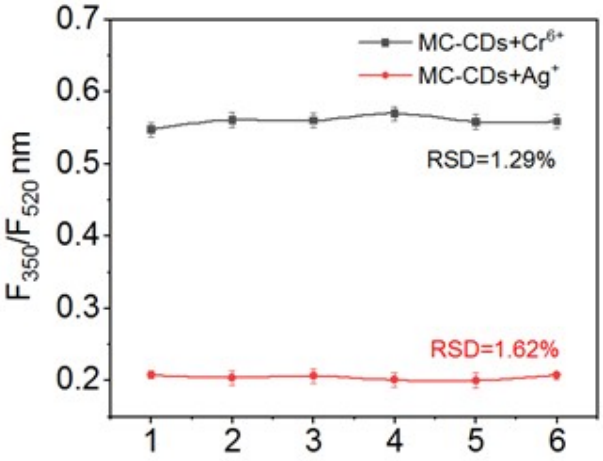

Fig. S8. The reproducibility of MC-CDs in detecting Ag^{+} and Cr^{6+}.

Table S2. Contents of Cr^{6+} and Ag^{+} in four TCMs analyzed by this method and compared to ICP-MS method.

Analytes	**Samples**	**Content (average±SD, RSD%, mg/kg) (n=3)**	
		This method	**ICP-MS method**
Cr^{6+}	*Dioscorea opposita* Thunb.1	0.127 ± 0.018 (1.91%)	0.129 ± 0.018 (1.56%)
	Dioscorea opposita Thunb.2	0.125 ± 0.004 (2.88%)	0.125 ± 0.005 (2.88%)
	Dioscorea opposita Thunb.3	0.120 ± 0.018 (1.91%)	0.124 ± 0.006 (1.94%)
	Crataegus pinnatifida Bge.1	0.132 ± 0.007 (2.13%)	0.141 ±0.002 (1.08%)
	Crataegus pinnatifida Bge.2	0.138 ± 0.002 (1.15%)	0.140 ± 0.003 (1.89%)
	Crataegus pinnatifida Bge.3	0.135 ± 0.003 (2.22%)	0.141 ± 0.002 (1.45%)

	Lycium barbarum L.1	0.124 ± 0.002 (2.36%)	0.120 ± 0.003 (2.54%)
	Lycium barbarum L.2	0.121 ± 0.002 (1.55%)	0.127 ± 0.002 (1.32%)
	Lycium barbarum L.3	0.121 ± 0.002 (2.25%)	0.126 ± 0.004 (2.28%)
	Lonicera japonica Thunb.1	0.093 ± 0.004 (1.67%)	0.099 ±0.002 (2.39%)
	Lonicera japonica Thunb.2	0.096 ± 0.008 (2.62%)	0.095 ± 0.003 (2.64%)
	Lonicera japonica Thunb.3	0.096 ± 0.007 (1.11%)	0.098 ± 0.003 (2.52%)
Ag^+	*Dioscorea opposita* Thunb.1	< LOD, ND	< LOD, ND
	Dioscorea opposita Thunb.2	< LOD, ND	< LOD, ND
	Dioscorea opposita Thunb.3	< LOD, ND	< LOD, ND
	Crataegus pinnatifida Bge.1	< LOD, ND	< LOD, ND
	Crataegus pinnatifida Bge.2	< LOD, ND	< LOD, ND
	Crataegus pinnatifida Bge.3	< LOD, ND	< LOD, ND
	Lycium barbarum L.1	< LOD, ND	< LOD, ND
	Lycium barbarum L.2	< LOD, ND	< LOD, ND
	Lycium barbarum L.3	< LOD, ND	< LOD, ND
	Lonicera japonica Thunb.1	< LOD, ND	< LOD, ND
	Lonicera japonica Thunb.2	< LOD, ND	< LOD, ND
	Lonicera japonica Thunb.3	< LOD, ND	< LOD, ND

ND: Not Detected.

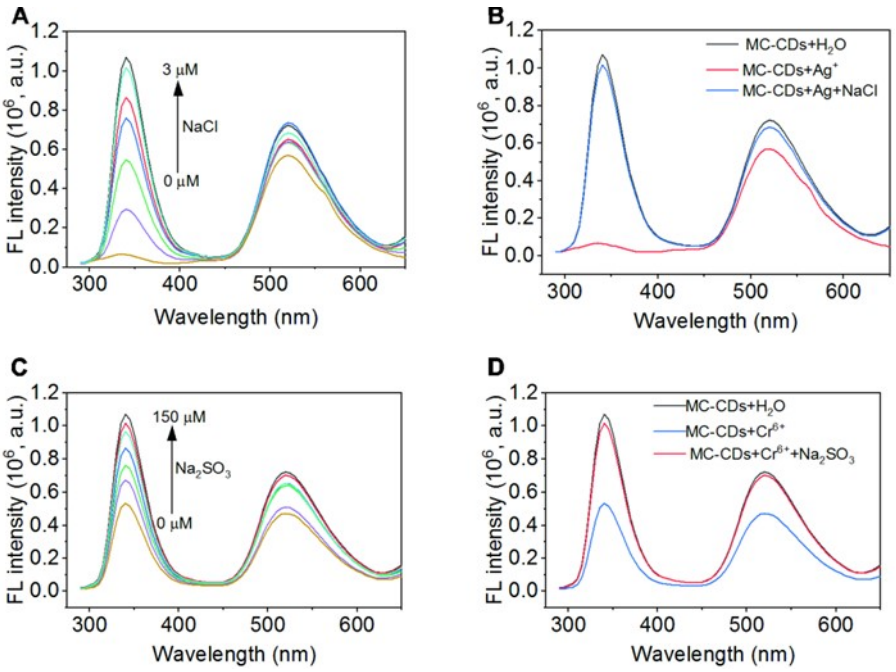

Fig. S9. The fluorescence recovery of MC-CDs-Ag^+ and MC-CDs-Cr^{6+} system upon the addition of NaCl and Na_2SO_3 within the range of 0-3μM and 0-150 μM.

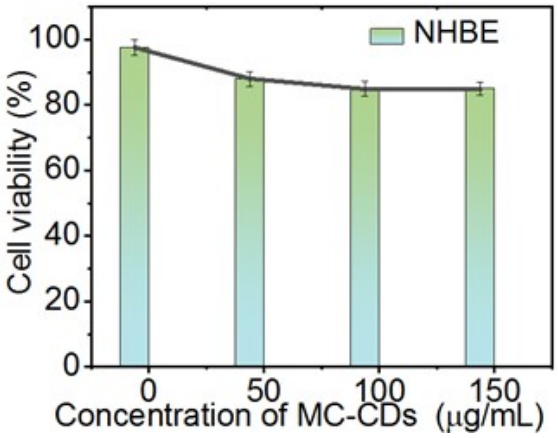

Fig. S10. Cell viability of NHBE cells following 24 h incubation with various concentrations of MC-CDs (0-150 μg/mL)

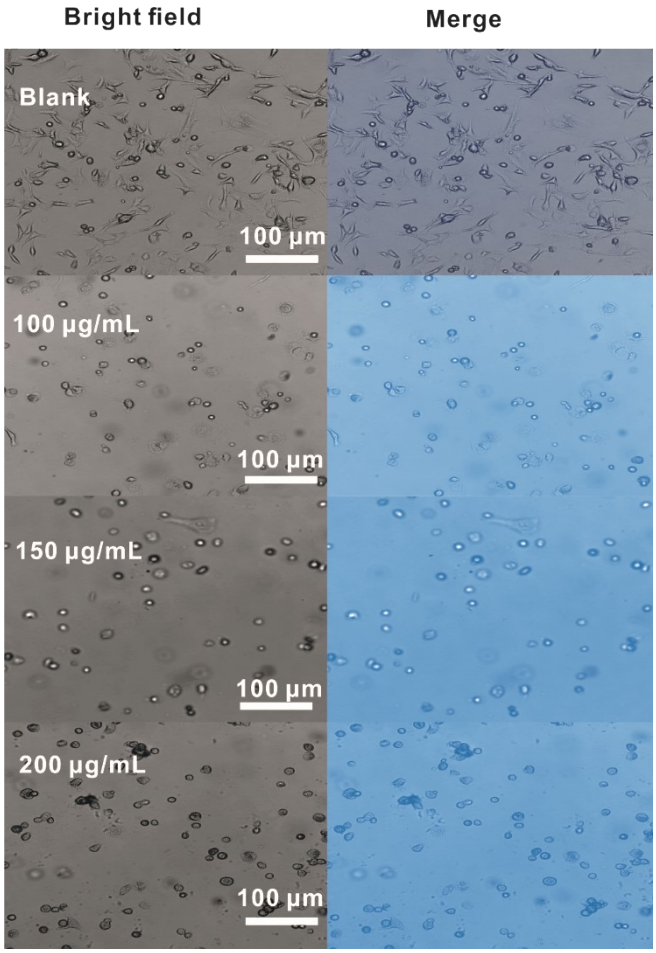

Fig. S11. Confocal microscopy images of A549 cells treated with 0, 100, 150, 200 μg/mL MC-CDs for 24 h under bright-field and merged channels.

Reference

1 Y. Huang, Z. Jiang, J. Wang, G. Yin, K. Jiang, J. Tu, T. Wang, Quality evaluation of mahonia bealei (Fort.) carr. using supercritical fluid chromatography with chemical pattern recognition, *Molecules*, 2019, ***24***, 3684.

2 Y. Cheng, F. Zhang, Y. Zhao, J. Chen, W. Yao, H. Qian, A "turn-on" fluorescence sensor for hydroquinone detection based on BSA doped carbon dots (BSA@CDs) from crawfish shells, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2025, ***325***, 125100.

3 Y. Mei, S. Yang, M. Su, J. Xie, S. Huang, Z. Jiang, Y. Huang, Nitrogen-doped carbon dots for ultrasensitive detection of Hg2 + in traditional Chinese medicine and bioimaging applications, *Analytical Methods*, 2025, ***17***, 5400-5411.