Electronic supplementary information

for

Multifunctional fluorescent carbon dot inhibits the invasiveness of lung cancer cells

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Experimental Details

Apparatus

The fluorescence of PEG400-CDs was measured with an F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) equipped with a 200μL microquartz fluorescence cell. TEM observations were carried out on a Hitachi JEM-2000 high-resolution transmission electron microscope, using an accelerating voltage of 200 kV. Samples were cast from water solutions onto copper grids by placing a droplet of a aqueous sample solution on grids. Fluorescence and bright field imaging was performed with a Eclipse Ti-S inverted fluorescent microscope (Nikon, Japan). Mili-Q purified water with a resistivity of 18.2 MΩ•cm was used throughout the experiments.

Cell Culture.

Non-small cell lung cancer (NSCLC) cell A549 and mice fibroblast L929 cells in the experiment were all stable cultured in our laboratory. The cells were cultured in DMEM high glucose medium (Hyclone) with 10% of Australia fetal bovine serum (GIBCO) in an atmosphere of 5% CO_2 at 37℃. 75 cm^2 culture flasks were selected and all cell sample were harvested in a solution of trypsin-EDTA at the logarithmic growth phase (GIBCO).

Proliferation assay

Approximately 5×10^3 cells were seeded separately in 96-well plates, incubated with various amount of CDs for 48 h, and treated with EnoGeneCell Counting Kit-8 (CCK-8) at 37 °C. After 2-3 hrs incubation, the cell viability was measured at 450 nm by an Microplate Reader (Thermo Scientific Multikcan Go, America).

Scratch test

3 groups of cells in logarithmic growth phase were seeded in 12-well cell culture plate. A trace on the surface of cells in culture was draw with a pipette tip, the scratches "healing" case was observed under phase contrast microscope after culturing with serum-free medium with different amount of nanoparticles for 24 hours and 48 hours. Scratch marks width and the number of cells within the distribution were measured. The experiment was repeated thrice.

Transwell migration assay

24-well Boyden chamber (Corning, Corning, NY, USA) was used for the cell migration assays. Briefly, A549 cells in logarithmic growth phase (70%-80% in integration of state) were seeded in the inserts at a density of 6×10^4 cells/well, insert that was coated with 60-μl diluted Matrigel (Matrigel: serum-free medium = 1:12). DMEM containing 10% fetal bovine serum (FBS) and CDs was placed in the lower chamber. After a 12-h co-culture, non-migrating cells on the upper surface of the membrane were erased using a cotton swab dipped in serum-free medium. Membrane was then immersed in 95% ethanol for 10min and stained by crystal violet. An inverted fluorescence microscope was chosen to observe cells penetrate the membrane of the lower chamber, counting five randomly chosen fields and per insert using Image J software. And then the fluorescent imaging.

Western blot analysis

Total protein was extracted from cultured cells with lysis buffer solution (EnoGene™, Nanjing, China). Afterward, the protein concentration was quantified using a BCA Protein Assay Kit (EnoGene™, Nanjing, China). Then, equal amounts of protein (Millipore, Boston, MA, USA) was subjected to electrophoresis on a 12% SDS–PAGE and transferred to a PVDF membrane (Millipore, Boston, MA, USA). Membranes were incubated with 5% non- fat dry milk in TBST and probed with anti-MMP9 andβ-actin in TBST (0.1% Tween 20 in TBS). Horseradish peroxidase-conjugated anti- rabbit IgG (Sigma) was used for detection of immunoreactive proteins by chemilum inescence (Western Blot Chemiluminescence Reagent Plus). All Western blot assays were repeated at least 3 times.

Real time quantitative PCR

Total cellular RNA was isolated from A549 cells (incubated 0 or 0.12 mol/L PEG400-CDs) cultured in 0.2% FBS DMEM using standard procedures, and retro-transcribed to cDNA. Quantitative PCR was performed with the gene specific primers of MMP-2 and MMP-9, using β-actin as the endogenous control. And the following cycling conditions were employed: denaturation, annealing, and extension at 95 °C, 57 °C and 72 °C for 10 s, 30 s, and 10 s, respectively, for 40 cycles.

Gelatin Zymography

A549 cells were seeded in 24 well plates and treated with or without PEG400-CDs for 24 h, and the conditioned media were collected to measure MMP-2 and MMP-9 activity. The medium was subjected to SDS-PAGE in an 8% polyacrylamide gel which was copolymerized with gelatin (0.08%).

Statistical analysis

Statistical analysis Data were expressed as means ± standard error (SEM). T tests was used to confirming normal distribution of the data. In all the groups, twoway analysis of variance (ANOVA) was used to determine differences among the groups. The values are expressed as means ± SEM. Data was analyzed using the Graph Pad prism software, version 5.0. $*p < 0.05$, $**p < 0.01$. Statistical differences were considered significant for values of $p < 0.05$.

Figures

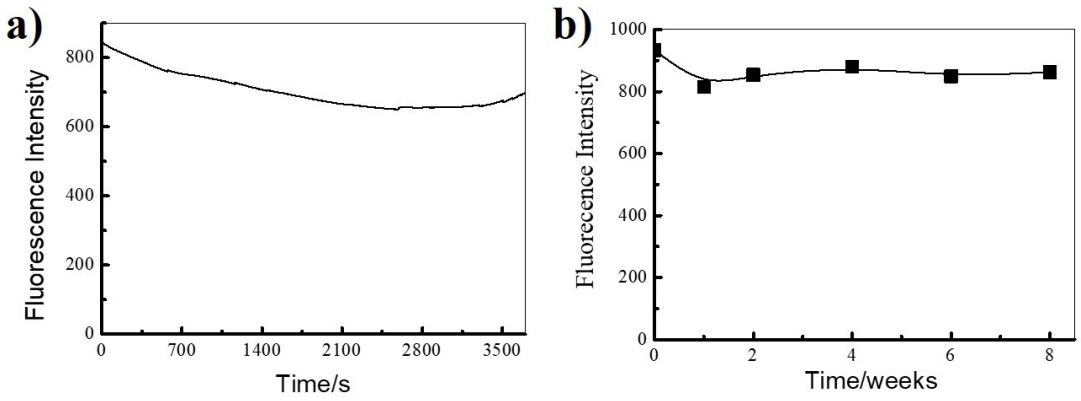

Fig. S1 The Photostability of PEG400-CDs continously excitted by Xenon lamp (285 nm) (a) or exposed to air (b).

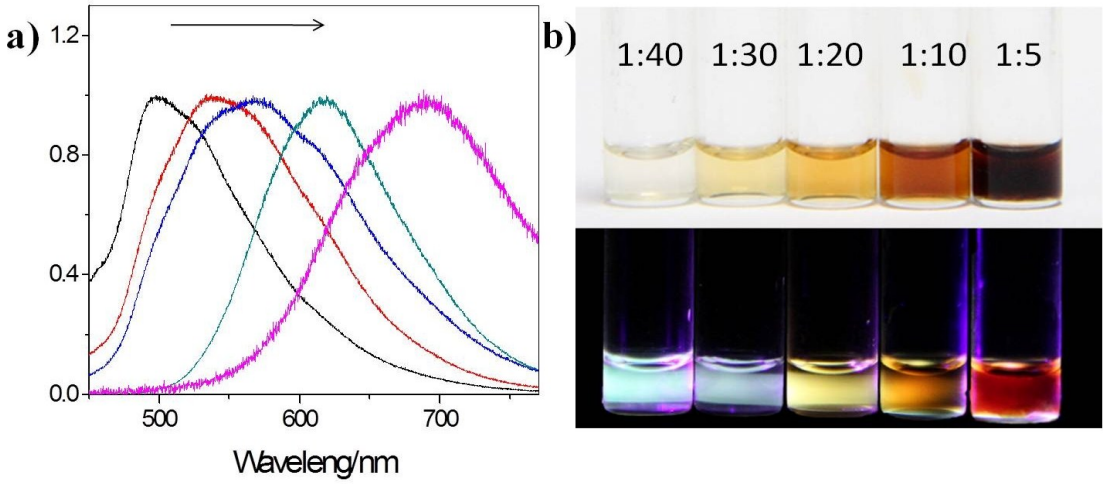

Fig. S2 a) Emission spectra of PEG400-CDs prepared under different ration of NaOH to PEG400 (from left to right: 1:40, 1:30, 1:20,1:10, 1:5); b) photographs taken under sunlight (Up) and 405nm UV lamp(Down).

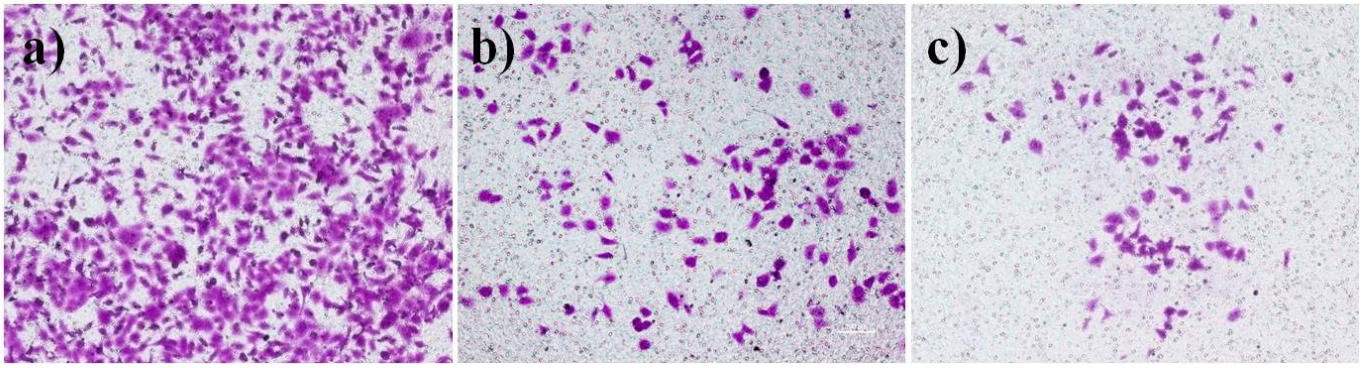

Fig. S3 Optimization of the matrigel dilution ratio a) no matrigel, b) 1:12, c) 1:6 for monitoring the cell invasion.

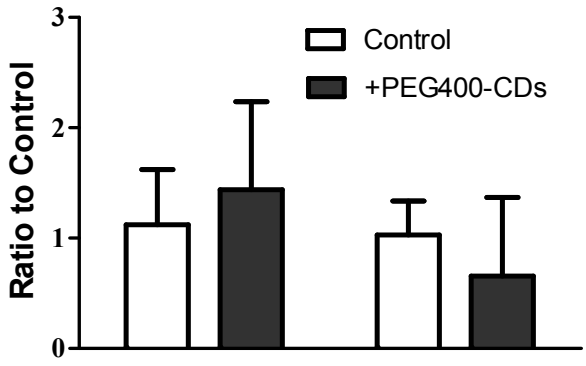

Fig. S4 A549 cells were treated with PEG400-CDs (0.12 mg/ml) for 24 h, qPCR was employed to examine the mRNA levels of MMP-2 and MMP-9.