Supporting Information

Biocompatible and low-cost iodine-doped carbon dots as bifunctional fluorescent and radiocontrast agent for X-ray CT imaging

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Materials and Methods

1.1 Drosophila maintenance and lines

Fly stocks were maintained at 18°C under standard conditions. In this work, we used reported fly lines to generate a fly line that contains fluorescence markers for non-muscle Myosin 2, spindle and membranes with the genotype as follow: w-; worGal4,Sqh:EGFP,UASCherry:Zeus/CyO;UASCaax:Cherry/TM6B [1, 2].

1.2 Live cell imaging of Drosophila 3rd instar larva brain

The first parental generation (G0), which gives rise to the second generation (G1), was kept in a plastic tube with fly food at 25°C for a full day. The G0 flies were taken out after 24 hours, and the plastic tubes with the laid embryos or eggs were maintained at 25°C until the larvae were 72–96 hours old. These third instar larvae were then dissected to obtain the brain lobes for time-lapsed

imaging of the neuroblasts. The larvae were dissected under a Zeiss Discovery V12 dissection stereoscope.

An adapted protocol was established to perform live cell imaging on intact larval brain lobes at room temperature. Briefly, *Drosophila* brains were dissected in Chan and Gehring medium (Chan & Gehring, 1971) and incubated with collagenase type I (Sigma Aldrich) at room temperature for 10 minutesat a final concentration of 476 µg/mL to remove the glial blood-brainbarrier. The brains were carefully washed three times with 200 µl of S2 supplemented medium. Brain lobes were then transferred to the Ibidi well containing 400 µl of S2 supplemented medium. For the I-CDs treated experiments, the amounts of I-CDs were added in the S2 supplemented medium accordingly to produce the final concentrations of either 50 ppm or 200 ppm.

For both the control and treated groups, live-cell imaging of the Drosophila intact brain lobes was conducted using the Leica Thunder Imager with a 63X objective (NA=1.4). The z-step size for a 30 µm thick z-stack was 1.25 µm, and the temporal resolution was set to 60 seconds. Representative image sequences of neuroblast diving in an intact brain were obtained using the ZEISS LSM 980 Airyscan 2 confocal microscope and a 63X objective (NA=1.4).

1.3 Image processing and quantifications

Live cell imaging data were processed using BitplaneImaris x64 10.0.1. All images and figures were generated using GraphPad Prism and Adobe Illustrator. Quantifications of ATPase activity were performed in Microsoft Excel.

1.4 ATPase activity assay

A malachite green assay was used to determine ATPase activity for five concentrations of I-CDs. I-CDs at concentrations of 50, 100, 200, and 10000 ppm were subjected to the malachite green assay using the Sigma-Aldrich ATPase/GTPase Activity Assay Kit (MAK113). Assays were performed according to the standard protocol of this kit at room temperature in triplicate. The kit includes a single reagent for determining enzymatic activity. This reagent reacts with the free phosphate released by the enzymes, forming a dark green color, resulting in a colorimetric product. The absorbance of this product was measured at 620 nm with an exposure time of 250 ms.

1.5 Cell culture and cytotoxicity assay

HeLa cells and normal human dermal fibroblasts (NHDFs, under 6th passage) were both purchased from American Type Culture Collection (ATCC, Manassas, VA) and routinely cultured in growth medium (GM) composed of Dulbecco's modified Eagle's medium (DMEM, Welgene, Daegu, Republic of Korea) supplemented with 10% fetal bovine serum (FBS, Welgene) and 1% antibiotic-antimycotic solution (Welgene, including 10,000 units penicillin, 10 mg streptomycin, and 25 mg amphotericin B per ml) at 37 °C in a humidified incubator with 5% CO2 atmosphere. Before cytotoxicity analysis, cells were subcultured at 80% confluency more than three passages after thawing for stabilization.The cell viability was tested using a cell counting kit-8 (CCK-8, Dojindo, Kumamoto, Japan) assay, including highly water-soluble tetrazolium salt [WST-8, 2-(2-methoxy-4-nitrophenyl)- 3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt], which is reduced to a yellow color formazan dye by mitochondrial dehydrogenases. Both cells were trypsinized by trypsin EDTA (Welgene) and seeded on the 96-well plate with the number of 1.5 × 104 cells/mL. After 24 h of culture, I-CDswere treated in the concentration of 0.2 – 200 μg/mL and further cultured in a humidified incubator for 24 h. Subsequently, cells were washed with Dulbecco's phosphate-buffered saline (DPBS) three times and reacted with diluted CCK-8 assay solution in GM (1:9, v/v) for 2 h. The 100 μL supernatants were moved to new 96-well plates and their absorbances were assessed using a microplate reader (Varioskan LUX, Thermofisher Scientific, Waltham, MA) at 450 nm. Cell viability was calculated by the percentage ratio of optical density compared to the control group (non-treated).

HEK293 (Human Embryo Kidney) and A549 (adenocarcinoma human alveolar basal epithelial) cells were placed in a 96-well plate at a density of 5×10^3 cells per well. The plate was then incubated at 37°C with 5% CO_2 overnight. The following day, suspensions of I-CDs in cell media (DMEM, 10%FBS, 1% penicillin/streptomycin) were introduced at concentrations of 0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.3, 12.5, 25, 50, 100 and 200 μg/mL. The samples were then incubated for 24 hours at a temperature of 37°C in a humidified atmosphere containing 5% CO_2. Cells that were not exposed to I-CDs were designated as the negative control. Following the application of I-CDs, 20 μl of MTT dye was introduced into each well to facilitate the conversion of the MTT tetrazolium component into formazan by the mitochondria of the cells. This process took place for 4 hours at a temperature of 37°C in a humidified atmosphere containing 5% CO_2. Subsequently, 100 μl of a 10% Sodium dodecyl sulfate (SDS) solution was added to dissolve the resulting formazan crystals.

The mixture was allowed to react overnight at room temperature, protected from light. The measurement of absorbance was conducted at a wavelength of 570 nm using a Microplate reader Varioscan (Thermo Scientific, USA). The viability was calculated using the following formula:

$$\%\ Cell\ Viability = \frac{Absorbance\ 570\ of\ treated\ cells}{Absorbance\ 570\ of\ control} \times 100\%$$

1.6 Fluorescence microscopy of mammalian cell lines

The Zeiss LSM980 Airyscan 2 confocal microscope with 63X objective (Carl Zeiss, Germany) was used to take bright field and green fluorescent images of livecells on an 8-well Ibidi glass bottom slide after 24 hours of incubation with 5000 ppm I-CDs. The images were captured using both bright-field (via T-PMT) and GFP (488 nm excitation) channels, with 1X zoom, 0.66 µs dwell time, and 2% laser power.The cells were not stained or treated with any dye or antibody. The green fluorescent signals detected are either the result of I-CD emission or autofluorescence in the case of untreated cells.

1.7 Statistical analysis

GraphPad Prism was used to conduct statistical analysis. All data were gathered from at least three independent experiments for each condition. One-way ANOVA test was used to determine statistical significance because all of the data were normally distributed. Statistical significance was indicated as follows: *, $0.01< p < 0.05$; **, $0.001< p < 0.01$; ***, $0.0001< p < 0.001$; ****, $p < 0.0001$; ns, not significant.

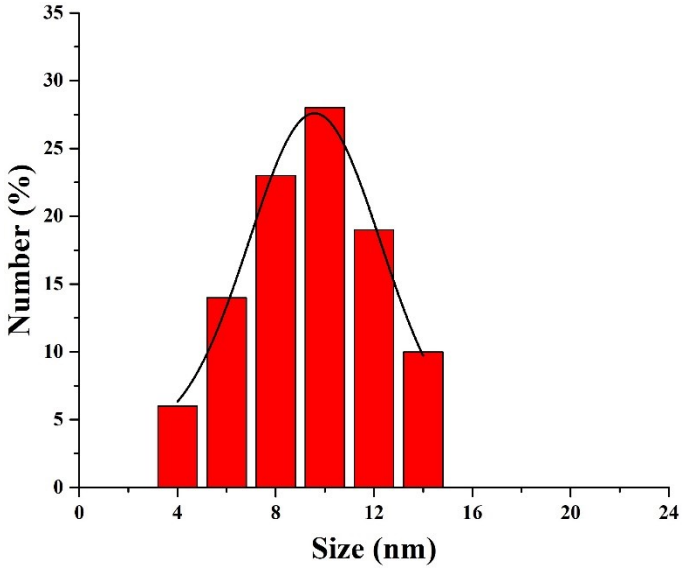

Figure S1. DLS analysis of I-CDs.

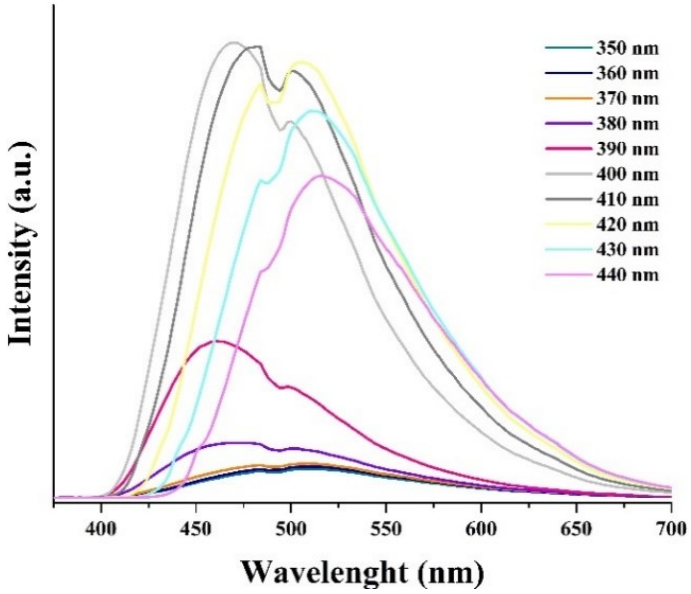

Figure S2. Excitation-dependent emission of prepared I-CDs.

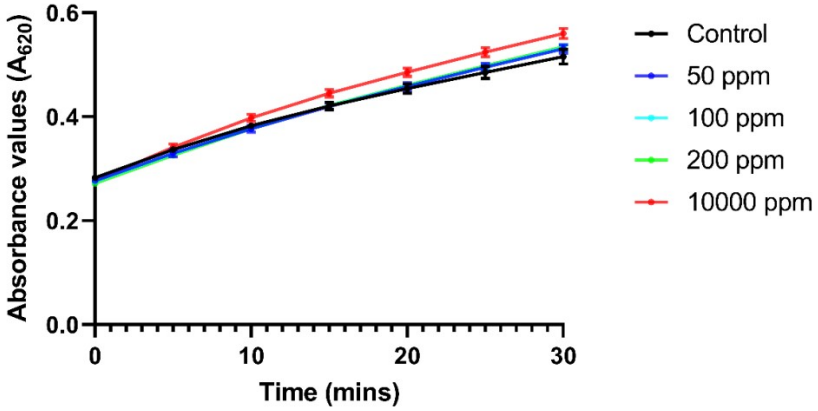

Figure S3. Absorbance values obtained from a colorimetric product of free phosphate using the ATPase/GTPaseactivity assay kit.

Table S1. ATPase activity values for different concentrations of I-CDs.

Concentration (ppm)	ATPase activity (nM/min/µL)
50	0. 95 ± 0. 0078
100	0. 95 ± 0. 0066
200	0. 95 ± 0. 0055
10000	0. 98 ± 0. 0094

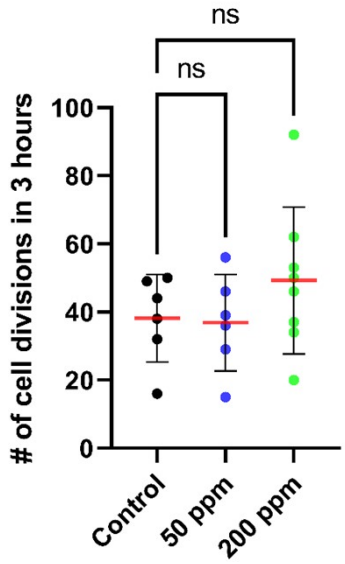

Figure S4. The number of cell divisions observed over three hours of live cell imaging. The statistical significance was determined usingone-way ANOVA.

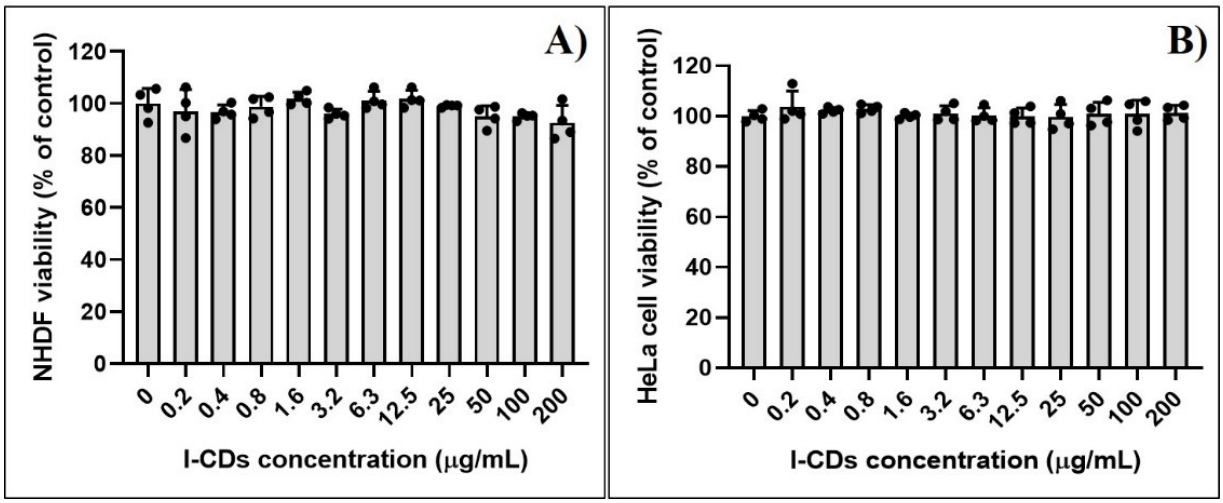

Figure S5. Effects of I-CDs on NHDF and HeLa cell lines viability after 24 hours (p value < 0.005)

References

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2) T.T. Pham, A. Monnard, J.Helenius, E. Lund, N. Lee, D. J. Müller, C.Cabernard. Spatiotemporally controlled myosin relocalization and internal pressure generate sibling cell size asymmetry. *iScience* 2019, 13, 9–19.