Electronic Supplementary Information

Sweet Nanodot for Biomedical Imaging: Carbon Dot Derived from Xylitol

Daeun Kim, Yuri Choi, Eeseul Shin, Yun Kyung Jung, and Byeong-Su Kim*

Department of Chemistry and Department of Energy Engineering,
Ulsan National Institute of Science and Technology (UNIST),
Ulsan 689-798, Korea

Experimental

Materials: Xylitol and 1,2-ethylenediamine (EDA) were purchased from SigmaAldrich. Hydrochloric acid (HCl) was purchased from Daejung Chemical (Korea).

Preparation of carbon dots (CDs): CDs were synthesized by a commercial household microwave (700 W). Firstly, 152.15 mg (1.0 mmol) of xylitol was diluted with 10 ml of DI water, and then mixed with different volumes (0 μL to 3 μL, 0 mmol to 3 mmol) of 1.0 M HCl, so that carbonization and surface passivation proceeded. Secondly, 68 μL of EDA (1 mmol) was mixed with solution to under vigorous stirring for 2 min. Then, the transparent solution was placed into a microwave oven and heated for 2 min. After cooling down to room temperature, the obtained yellow-brown solid was dissolved into DI water and filtered with a syringe filter (0.45 μm) to remove salt and unreacted residues. Finally, the solution was dialyzed against DI water through a dialysis membrane (MWCO of 500 - 1000) for 1 day. To observe the influence of EDA, CD_{xy} was synthesized by the identical procedure with 1 ml of 1 M HCl (1.0 mmol) at a different amount of EDA (0 μL to 204 μL, 0 mmol to 3 mmol).

Characterization: UV/vis spectrophotometer (UV-2550, Shimadzu) was used to record absorbance for concentration control and comparison. Absorbance curves were compared to ascertain the similarity in concentration, after which samples were adjusted for comparison. Fluorescence data was obtained by fluorometer (Agilent). Transmission electron microscopy (TEM, JEM-2100, JEOL) and atomic force microscopy (AFM, Dimension 3100, Veeco) were performed to investigate the size and morphology of the CDs. To confirm the functional groups after passivation, XPS (K-alpha, Thermo Fisher), FT-IR (Agilent) and Zeta potential measuring system (Malvern) were performed.

Photoluminescence lifetime measurement: The exciton lifetime was determined by the time-correlated single photon counting (TCSPC) technique. The computer controlled diode laser with 375 nm wavelength, 54 ps pulse width and 40 MHz repetition rate was used as an excitation source. The PL emission was spectrally resolved by using some collection optics and a monochromator (PicoQuant). The TCSPC module (PicoHarp 300E, PicoQuant) with a MCP-PMT (R3809U-5x series, Hamamatsu) was used for ultrafast detection. The total instrument response function (IRF) for PL decay was less than 30 ps, and the temporal time resolution was less than 10 ps. The deconvolution of actual fluorescence decay and IRF was performed by using a fitting software (FlouFit, PicoQuant) to deduce the time constant associated with each exponential decay.

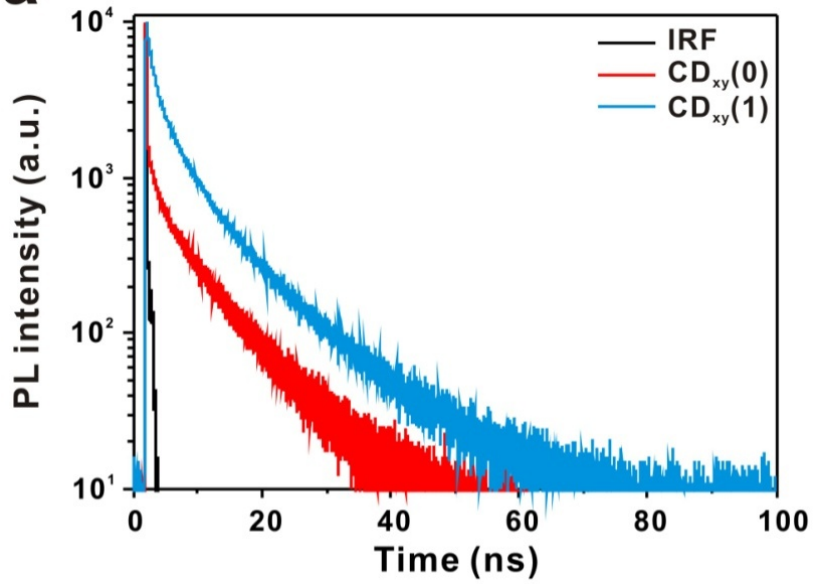
Cell culture: HeLa cells, derived from human epithelial carcinoma cells, were incubated with Dulbecco's Modified Eagle's Medium (DMEM, Life technologies) with 10% fetal bovine serum and 1% penicillin-streptomycin. WI-38 cells, derived from human diploid cells, were incubated with Roswell Park Memorial Institute (RPMI) 1640 media (Life Technologies) with 10% fetal bovine serum, 25 mM sodium bicarbonate and 1% penicillin-streptomycin.

Cytotoxicity test: HeLa and WI-38 (human diploid cells) were purchased from the Korean Cell Line Bank (Seoul, Korea). Cell viability was assessed by the MTT assay. Cells were seeded in 96-well plates at a density of 1×10^4 cells per well and incubated for 24 h in 5% CO_2 at 37 °C. After removing the culture medium, the wells were washed with PBS. Each well was then replaced with 90 μL of fresh medium and 10 μL of 10X CDs solution. After 24 h in 5% CO_2 at 37 °C, thiazolyl blue tetrazolium bromide (MTT, SigmaAldrich) was added to each well of cells (final conc. 0.5 mg/mL) and incubated for 4 h in incubator. 100 μL of DMSO was added to solubilize the MTT-formazan product and the sample was incubated for further 15 min at room temperature. Absorbance of the solution was read at a test wavelength of 540 nm.

Cell imaging: Fluorescence imaging of cells was obtained using confocal laser scanning microscopy (CLSM, FV1000, Olympus America Inc.) at a magnification of ×1000. HeLa cell

was seeded into the 8-chambered cover glass at a density of 2 × 10^4 cells per well and incubated for 24 h in 5% CO_2 at 37 °C. After removing the culture medium, the wells were washed with PBS. Each well was then replaced with 180 μL of fresh medium and 20 μL of 10X CDs solution. After additional 24 h incubation, optical and fluorescence imaging was carried out under bright field, ultraviolet (405 nm), blue (473 nm), and green (559 nm) laser excitation, respectively.

a



b

	$CD_{xy}(0)$	$CD_{xy}(1)$
t1 (ns)	10.36 (0.11)	11.93 (0.09)
t2 (ns)	3.08 (0.18)	3.74 (0.31)
t3 (ns)	0.14 (0.71)	0.67 (0.60)
X^2	1.00	1.08
$t_{average}$ (ns)	1.75	2.63

Fig. S1 (a) Time-resolved photoluminescence decay curves. (b) Exciton lifetime of $CD_{xy}(0)$ and $CD_{xy}(1)$.

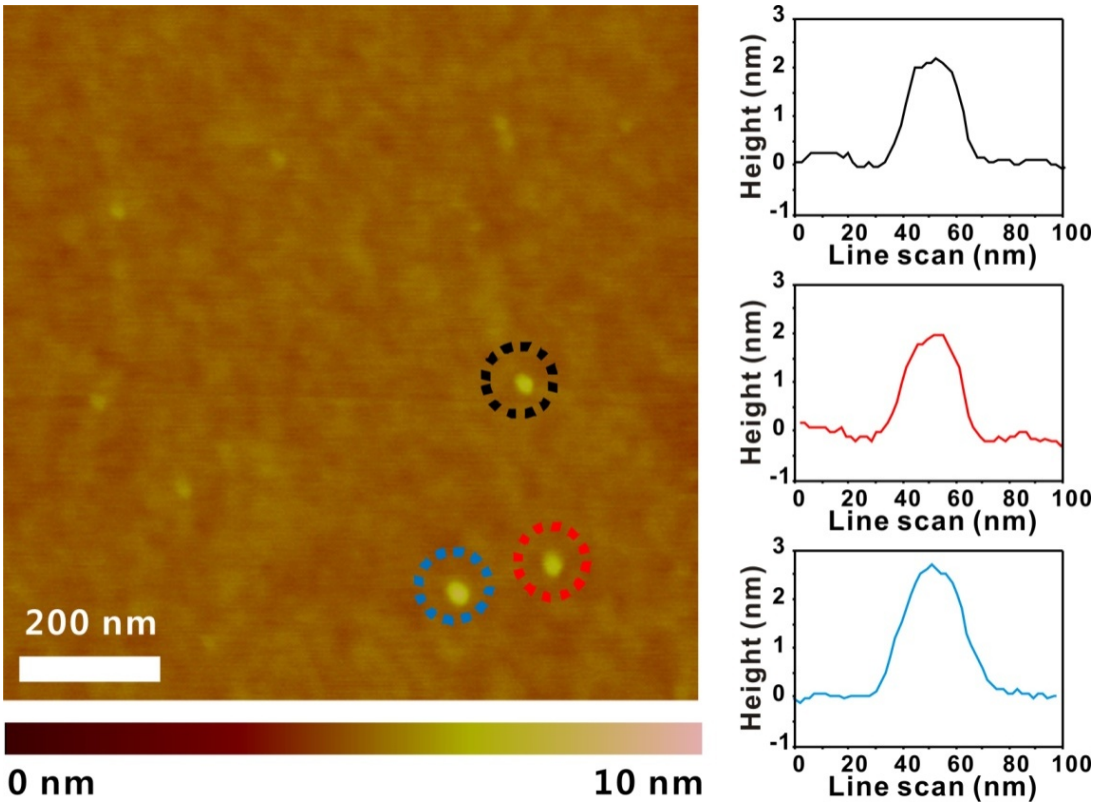


Fig. S2 Height-mode AFM image of $CD_{xy}(0)$ with corresponding line scan profiles.

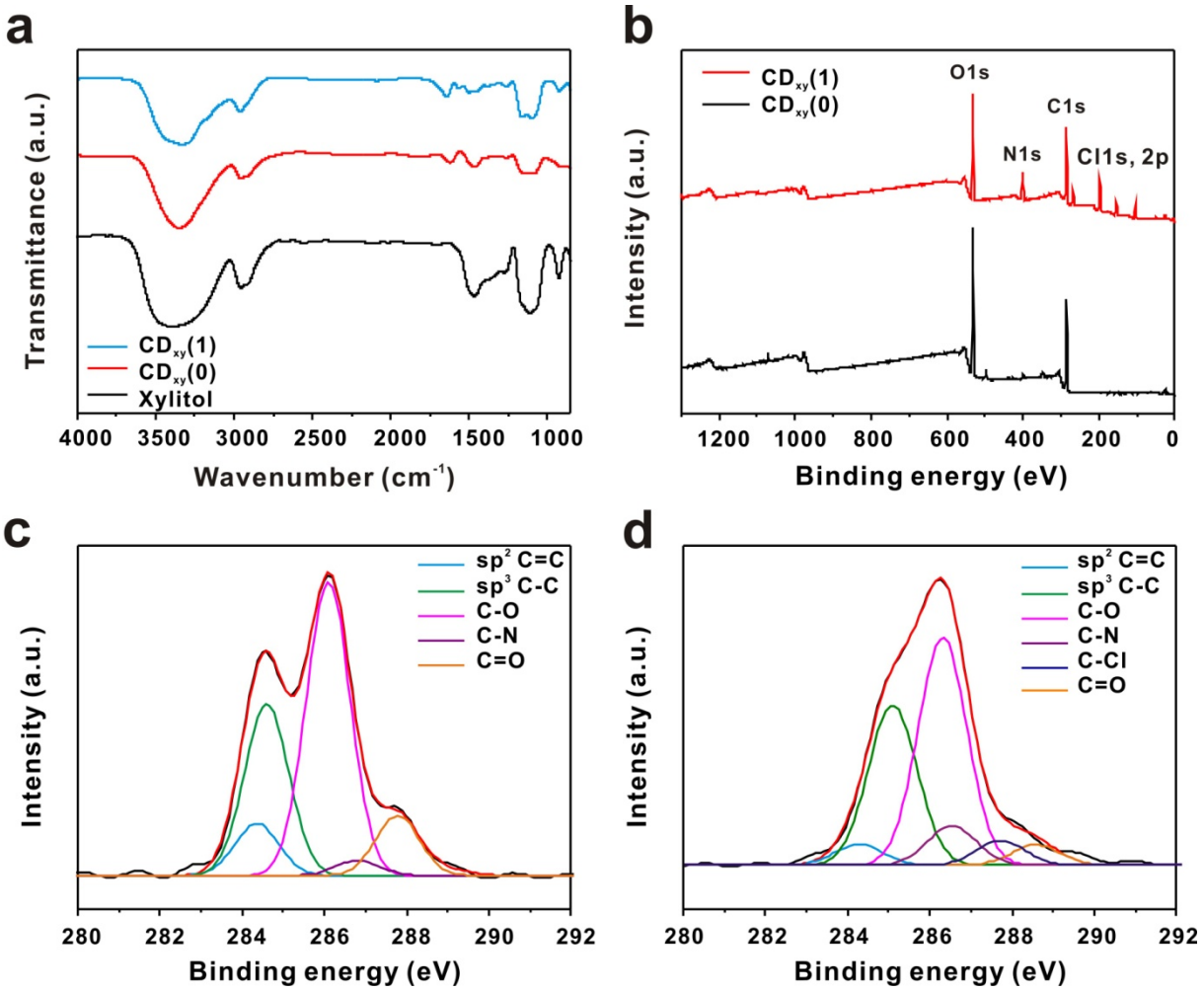


Fig. S3 (a) FT-IR spectra of xylitol, $CD_{xy}(0)$, and $CD_{xy}(1)$. (b) XPS survey spectra of $CD_{xy}(0)$ and $CD_{xy}(1)$. (c, d) The deconvoluted high-resolution XPS C1s spectra of (c) $CD_{xy}(0)$ and (d) $CD_{xy}(1)$. High resolution C1s spectra of $CD_{xy}(0)$ show five distinct components: sp^2-carbons (284.3 eV), sp^3-carbons (284.6 eV), C-O (286.1 eV), C-N (286.8 eV), and C=O (287.8 eV). And high-resolution C1s spectra of $CD_{xy}(1)$ show six distinct components: sp^2-carbons (284.2 eV), sp^3-carbons (285.0 eV), C-O (286.2 eV), C-N (286.5 eV), C-Cl (287.6 eV), and C=O (288.5 eV).

Table S1. XPS element composition analysis of CD_{xy}

Element [%]	C	O	N	Cl	N/C	O/C
$CD_{xy}(0)$	64.60	33.59	1.81	-	0.028	0.52
$CD_{xy}(1)$	52.51	25.69	12.38	9.42	0.24	0.49

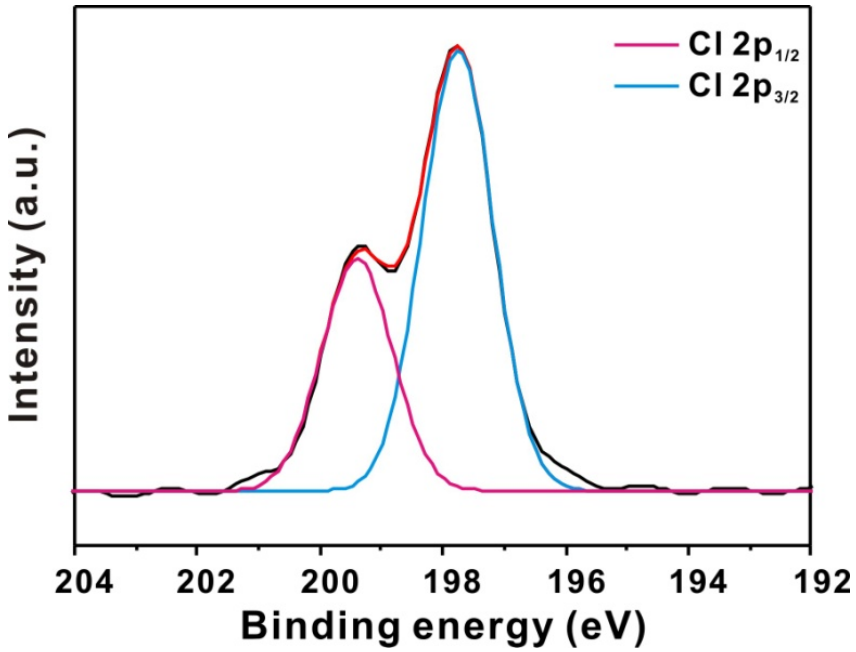


Fig. S4 Deconvoluted high-resolution XPS Cl 2p spectrum of $CD_{xy}(1)$.

Table S2. XPS Cl composition analysis of $CD_{xy}(1)$

$CD_{xy}(1)$	Position [eV]	Area [%]
Cl $2p_{1/2}$	199.4	34.43
Cl $2p_{3/2}$	197.7	65.56

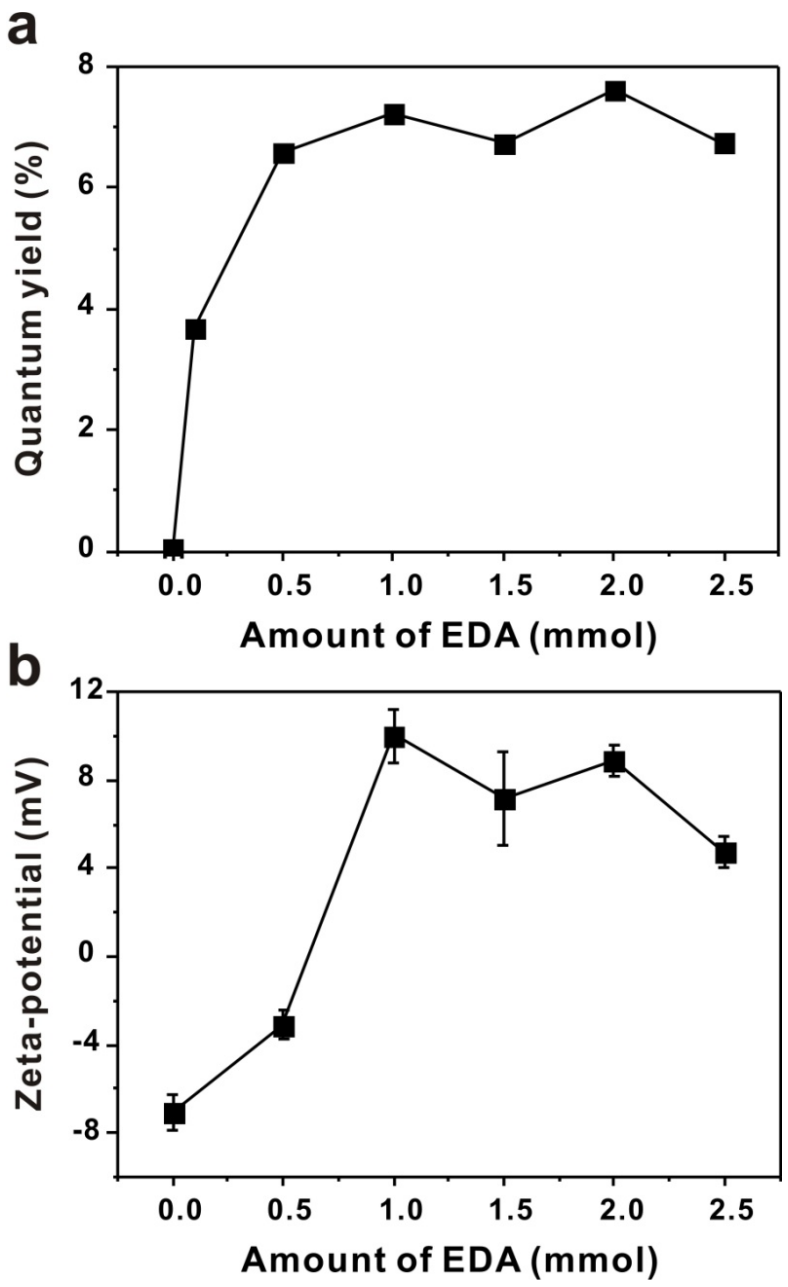


Fig. S5 (a) Quantum yield and (b) zeta-potential of CD_{xy} with varying amount of EDA. The amount of both xylitol and HCl was fixed to 1.0 mmol. Quantum yield was measured using a quinine sulfate as a reference at excitation wavelength of 360 nm.

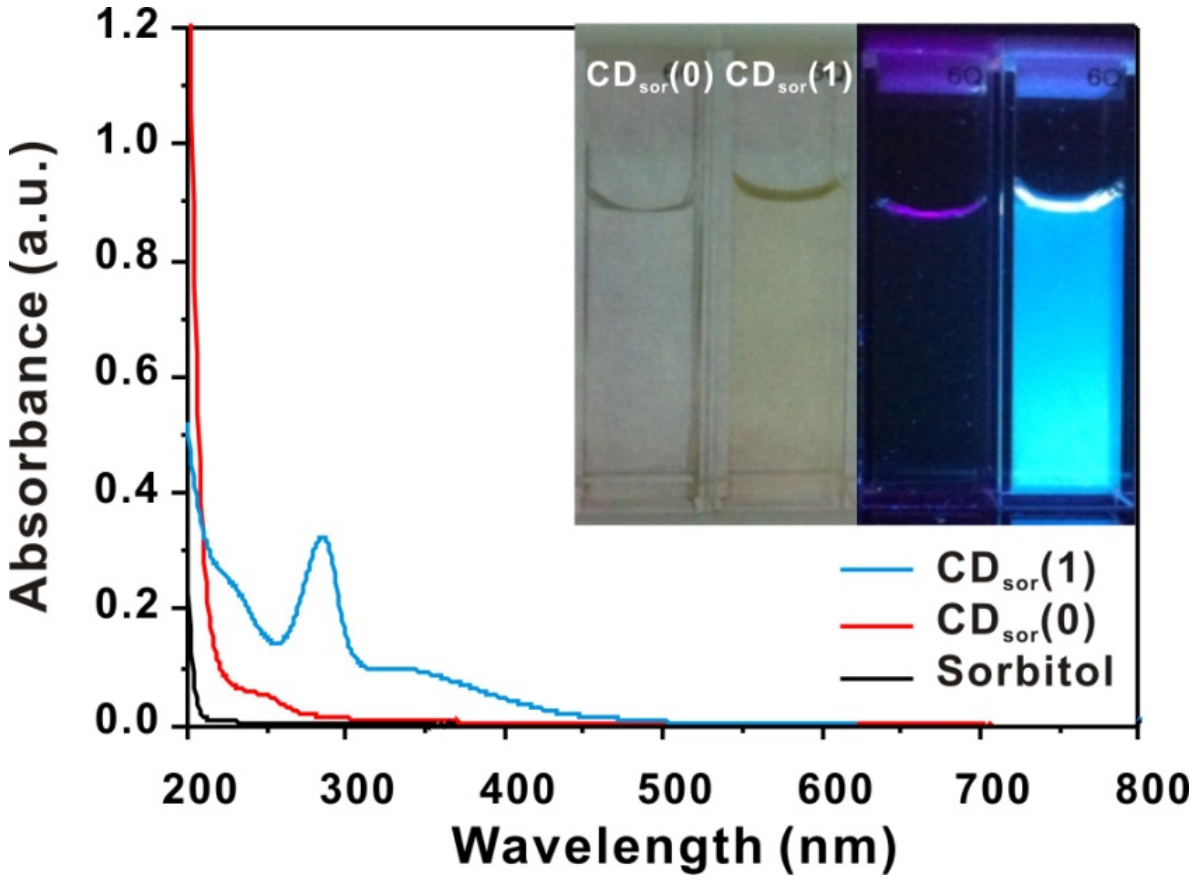


Fig. S6 UV/vis absorption spectra of sorbitol, $CD_{sor}(0)$ and $CD_{sor}(1)$. Inset shows (right) $CD_{sor}(0)$ and (left) $CD_{sor}(1)$ solution under daylight and UV light (365 nm). Quantum yields are determined to be 2.3% and 5.3% for $CD_{sor}(0)$ and $CD_{sor}(1)$, respectively.