Electronic Supplementary Information

Carbon Nanodots Directly Generated on Screen Printed Carbon Electrode-based Chip via Electrochemical Exfoliation and the Applications for Efficient Cell Imaging and Electrochemiluminescence Enhancement

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1. Experimental

S1.1 Chemicals

Chromatography paper (Whatman, No. 1) used for paper based Chips (PCs) was from Whatman International Ltd. (U. K.). SU-8 3025 negative photoresist was purchased from MicroChem Corp. (Newton, MA). Carbon ink and silver conductive ink were purchased from Alfa Aesar (MA, USA). The IL 1-ethyl-3-methylimidazolium tetrafluoroborate (IL, min. 98 %) used in this work were synthesized following the procedure described elsewhere. [1, 2] The starting materials were obtained from Sigma-Aldrich (Steinheim, Germany). HeLa cells were obtained from the American Type Culture Collection (Manassas, VA) and maintained in DMEM supplemented with 10% standard fetal bovine serum (HyClone Laboratories, UT) at

37 °C and in 5% CO_2. 35 mm glass chamber slides were purchased from Hangzhou Sanyou Biotechnology Co. Ltd. (Hangzhou, China). Tris(2,2'-bipyridyl)ruthenium(II) chloride hexahydrate [$(Ru(bpy)_3Cl_2 \cdot 6H_2O$, 98%] and Nafion (20 wt%) and quinine bisulfate were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI). Stock solutions of 100 mM phosphate buffered saline (PBS) (pH=7.4) was also prepared. All the other reagents were of analytical grade and Millipore Mill-Q water (18.2 MΩ cm) deionized water was used throughout.

S1.2 Apparatus and measurements

Static potentials of 0-30 V were applied to the two electrodes using a DC power supply PS-3005D. Electrochemical experiments were performed with a CH Instruments 800 voltammetric analyzer. Electrochemiluminescence (ECL) intensities were measured with a computer controlled CE-ECL system (Xi'an Remex Electronics Co. Ltd. Xi'an, China). The voltage of photomultiplier tube (PMT) for collecting the ECL signal was set at 800 V in the process of detection. X-ray photoelectron spectra (XPS) were collected on an Escalab 250 X-ray photoelectron spectroscopy with an Mg KX-ray source. The data were obtained at room temperature and typically the operating pressure in the analysis chamber was below 1×10^{-9} Torr. A XL30 ESEM scanning UV/vis absorption spectra were collected by a CARY 500 UV/vis/near-IR spectrophotometer (Varian Inc., USA). Fluorescence measurements were recorded at room temperature using a LS 55 luminescence spectrometer (Perkin-Elmer Instruments, U.K.). Transmission electron microscopy (TEM) images were obtained with a FEI TECNAI G2 transmission electron microscope (Eindhoven, Netherlands) operating voltage of 120 kV. The samples for TEM characterization were prepared by placing a drop of sample solution on carbon-coated copper grid and dried at room temperature. FTIR was obtained using Tensor 27 produced by Bruker Corporation.

S1.3 The intracellular uptake of CNDs, bioimaging and MTT assays

HeLa cells (10^6 cells per sample) were plated onto 35 mm glass chamber slides. 10% of the original CNDs solution in DMEM were then freshly prepared and placed over the cells for 1 h at 37 ◦C. All cells were washed with PBS buffer three times at room temperature to remove free and physically absorbed CNDs. After that, the fluorescence images of the cells were

taken using the LEICA TCS SP2 Laser scanning confocal microscope (LSCM, Leica Microsystems Heidelberg GmbH, Germany) with a 100 × oil immersion objective. The CNDs cultured cells were excited by using 488 nm Ar laser. [3]

MTT assays were used to evaluate the CNDs doses on the viability of the HeLa cells. The cells were treated with various concentrations of CNDs (0%, 2%, 5%, 10%, 20%, 100% of the original CDs solution, respectively) in fresh DMEM for 24 h. Treated cells were added with DMEM containing MTT (10 mL, 5 mg mL^{-1}) and further incubated at 5% CO_2, 37 ◦C for 4 h. Then, the MTT containing medium was added with 100 μL DMSO to solubilize the formazan crystals precipitate. Viability of untreated control cells was arbitrarily defined as 100%. Finally, the absorption at 490 nm of each well was measured by an EL808 ultramicroplate reader (Bio-TEK Instrument, Inc., Winooski, VT, USA). [3]

S1.4 Fabrication of the chips, SPCEs and the solid-state ECL sensor on PCs

The paper-based chips (PCs) were fabricated by photolithography according to previously reported method. [4, 5] Briefly, a piece of Whatman chromatography paper was soaked with SU-8 3025 photoresist. After baking at 95 °C for 5 min, the paper was exposed to UV light for 6 s through a photomask. The unpolymerized photoresist was removed by soaking the paper in acetone for 1 min and rinsing the paper with acetone for three times. Then, positive electrode (carbon) and negative electrode (carbon) were screen-printed on the PCs. The whole process can be finished within 10 min, and six or more chips can be produced once a time, indicating that this design was suitable for mass production. Glass slide substrates (75 mm × 25 mm) can also be used as chips, on which the same procedure was applied to fabricate the SPCEs.

The solid-state ECL sensors on PCs were prepared following the same procedure described previously. [5] A sample of 1.0 wt% Nafion solution was dispersed in the same volume of the ethanol, or IL or original CNDs with ultrasonication for 20 min to obtain the homogeneous, well-dispersed mixture solutions of pure Nafion, Nafion/IL and Nafion/CNDs, respectively. [5] The working SPCEs on three PCs were modified by dripping with the same volume (15 μL) the pure Nafion, Nafion/IL and Nafion/CNDs separately, and the solvents were allowed to evaporate under an infrared lamp heating for 60 s. Volume of 20 μL 5.0 mM $Ru(bpy)_3^{2+}$ aqueous solution was then pipette on the modified electrodes for incorporation of $Ru(bpy)_3^{2+}$

for 1 h. The prepared solid-state ECL sensors were rinsed thoroughly with deionized water to remove the unimmobilized $Ru(bpy)_3^{2+}$, followed being scanned in 100 mM PBS (pH 7.4) until the reproducible CVs and ECL signals were obtained. Due to the opaqueness of the SPCE, the side with the working and counter electrodes were placed over against the PMT to collect the utmost ECL signal.

References

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2. Results and Discussion

2.1 XPS spectra and FTIR spectrum of the as-prepared CNDs after being dialyzed for 72 hours.

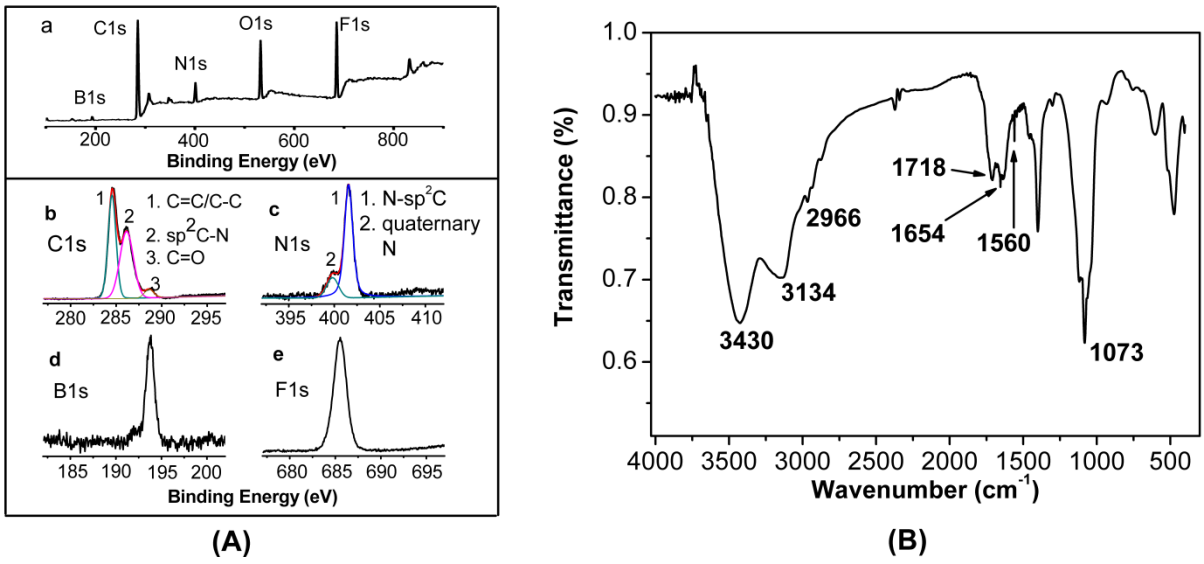

Fig. S1 (A) XPS spectra: (a) Survey XPS data; XPS spectra of (b) C 1s; (c) N1s; (d) B1s and (e) F1s, respectively; (B) FTIR spectrum of the as-prepared CNDs after being dialyzed for 72 hours.

2.2 Laser scanning confocal microscope images

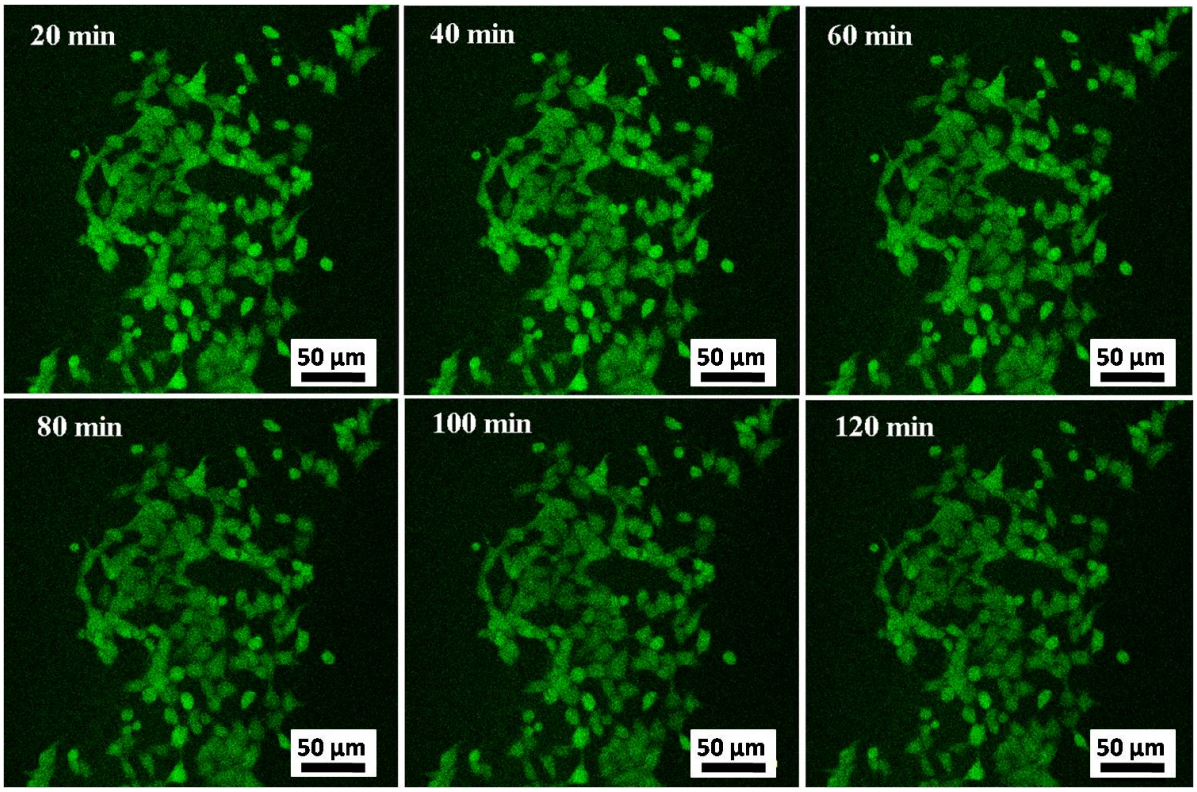

Fig. S2 LSCM images showing time-dependent fluorescence signals of Hela cells treated by the as-prepared CNDs.

2.3 Tripropylamine analysis using the Nafion/CNDs modified ECL sensor

For the tripropylamine (TPA) analysis using the Nafion/CNDs modified ECL sensor, standard curve was obtained from the analysis of TPA with different concentrations from 0.1 nM to 1 mM. The standard curve was linear in the range of 5.0 nM to 20 µM. The calibration equation and regression coefficient were: $y = 0.12502\ x + 3.79738$ and $R = 0.998$ ($n = 8$) in terms of log (ECL intensity, a.u.) as a function of log (TPA concentration, nM). Detection limit of 2.0 nM for TPA was achieved ($S\ N^{-1} = 3$).