Multicolor Fluorescent Graphene Oxide Quantum Dots for Sensing Cancer Cell Biomarkers

Lisandro Cunci[a,*], *Viviana González-Colón*[b], *Brenda Lee Vargas-Pérez*[a], *Joed Ortiz-Santiago*[a], *Miraida Pagán*[a], *Paola Carrion*[a], *Jomari Cruz*[a], *Agustin Molina-Ontoria*[c], *Namyr Martinez*[b], *Walter Silva*[b], *Luis Echegoyen*[d], *Carlos R. Cabrera*[e]

[a] Department of Chemistry, Universidad Ana G. Méndez, Carr. 189, Km 3.3, Gurabo, Puerto Rico 00778, United States

[b] Department of Physiology, University of Puerto Rico – Medical Sciences Campus, San Juan, Puerto Rico 00936, United States

[c] IMDEA-Nanociencia, C/Faraday, 9 Ciudad Universitaria de Cantoblanco, 28049 Madrid, Spain

[d] Department of Chemistry, University of Texas at El Paso, El Paso, Texas 79968, United States

[e] Department of Chemistry, University of Puerto Rico – Rio Piedras Campus, 17 Ave. Universidad STE 1701, 6, San Juan, Puerto Rico 00925, United States

Corresponding Author

* Tel.: +1-787-743-7979 x9-4744; fax: +1-787-743-4114; E-mail address: cuncil1@uagm.edu (L. Cunci).

Supporting Information

Experimental Section

Pristine onion-like carbon nanoparticles

The synthesis of the onion-like carbon nanoparticles was done following previously published procedures with few modifications.[1-2] Briefly, diamond nanoparticles (1 g) were placed in a combustion boat of high purity alumina and located at the center of a high temperature tube furnace. Then, the tube is sealed at the ends to avoid oxygen to enter and react with the diamond nanoparticles to produce CO_2, which would lose the sample. The tube was purged three times alternating helium and vacuum using a mechanical pump, and at the end, helium was left flowing at a maximum of 100 cc/min with a water seal on the other side of the tube to ensure that oxygen was not entering. After the preparation of the sample inside the tube furnace is done, the temperature ramp is selected at 5 °C/min up to 800 °C, and 2 °C/min up to 1,650 °C where the temperature is maintained. We tested different times and the difference is the structure seen using XRD obtained at 30 mins and 2 hours. After maintaining the temperature at 1,650 °C, the furnace was cooled down at a rate of 2 °C/min up to 800 °C, and the power was turned off until the furnace reaches room temperature when it was opened to obtain the CNO. If the synthesis was correctly done, the grey powder (diamond nanoparticles) should have turned completely black (CNO). About 976 mg of pure CNOs was obtained (98 % yield).

Graphene oxide quantum dots synthesis

Once CNO were synthesized, we used a procedure modified from a synthesis designed to obtain graphene oxide from graphite nanofibers in previous published work[3]. The procedure shown in Figure 1 in the manuscript started by (1) dispersing CNOs in a mixture of concentrated sulfuric acid and phosphoric acid (9:1) in a sonic bath. Then, the slurry was agitated using a magnetic stirrer while six times the mass of the CNO was added of $KMnO_4$ very slowly. (2) The mixture was left for 24 hours at 50 °C using a magnetic stirrer. Then, (3) the mixture was centrifuged for 10 mins at 15,000 rpm, which separated the acid solution from the nanoparticles. (4) The supernatant was separated and stored, while (5) the nanoparticles were mixed with a concentrated solution of NaOH, and sonicated until dispersion, which separated the GOQD from the inner cores. The separation of GOQD and OLIC was due to the deprotonation of the oxygenated functionalities to have a negative charge and repel each other. (6) The mixture was centrifuged again at 15,000 rpm to have the GOQD in the supernatant, and the inner cores precipitated. (7) GOQD was saved in a container where they were accumulated during the iteration. (8) The inner cores were mixed again with the acid mixture saved in step (4), and the cycle started again from step (2).

Graphene oxide quantum dots purification

The synthesis was finished when no more quantum dots were obtained from the separation, using as criteria the disappearance of the fluorescence seen when exciting the solution with a 365 nm light. The solution obtained was neutralized to a pH of ca. 7.0. Then, GOQD were purified by placing 50 ml of the GOQD solution in a 5,000 Da dialysis membrane tube in a 2 L container with

Millipore nanopure water (18.2 MΩ) which was changed twice a day for four days. After the purification, the solution was frozen using liquid nitrogen, and lyophilized to obtain the quantum dots as powder.

Graphene oxide quantum dots characterizations

Characterization of graphene oxide quantum dots was done using High Resolution Transmission Electron Microscopy with a JEOL JEM-2200FS HRTEM. Fluorescence was measured using a UV transilluminator with a 365 nm light. Fluorescent measurements were obtained using a dedicated Fluorecence Spetrophotometer as well as a Tecan Infinite M200 PRO Microplate Reader. UV-Vis absorption was measured using a UV-2401 Spectrophotometer from Shimadzu. Atomic force microscopy (AFM) was performed using a Bruker AFM MultiMode 8 with ScanAssyst. Raman spectroscopy was performed using a DXR 3 Raman imaging microscope from ThermoFisher Scientific.

Positive and negative extracts preparation

Jurkat T-Cells (American Type Culture Collection of Manassas, VA, USA) were incubated at 37 °C with 5% CO_2 using RPMI-1640 media (Hyclone) complemented with 10% Fetal Bovine Serum (Hyclone) and an antibiotic/antimycotic that consisted of penicillin, streptomycin and amphotericin B (Sigma). Peripheral blood mononuclear cells (PBMC) were obtained from a blood sample using Histopaque 1077 (Sigma-Aldrich) following the directions provided by the manufacturer and resuspended in the same media prepared for the Jurkat cells stated above.

Nuclear cell extract of both group of cells, Jurkat cells and PBMC was done using the procedure published by the authors[4] as follows: $1x10^6$ cell per mL were harvested and washed twice with cold 1 PBS (140 mM NaCl, 2.7 mM KCl, 10 mM $NaHPO_4$, and 1.8 mM KH_2PO_4) and centrifuged at 1500 rpm for 5 min. The pellet was resuspended in buffer A (10 mM HEPES–KOH [pH 7.9], 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT, and 1 mM PMSF). Then, the solution was placed on ice for 10 min and centrifuged at 1500 rpm for 10 min at 4 °C. Fresh buffer A was added to the pellet and lysed in a 7 mL Dounce homogenizer. The lysate was centrifuged at 6500 rpm for 2 min at 4 °C to separate the nuclei (pellet) from the cytoplasm (supernatant). The nuclear fraction was resuspended in 1 mL of the buffer C (20 mM HEPES [pH 7.9], 0.42 M NaCl, 0.2 mM EDTA, 25% glycerol, 1.5 mM $MgCl_2$, 0.5 mM PMSF and 0.5 mM DTT) and a sonifier was used to disrupt the fraction by sonicating 6 times for 10 s at maximum power. The nuclear fraction was centrifuged at maximum rpm for 30 min at 4 °C. Then, the nuclear extract was stored at -80 °C

Cytotoxicity

Cell Culture – Human Astrocytoma Cell Line 132N1 (ECACC#86030402, obtained from Sigma-Aldrich St. Louis, MO), were grown at 37 °C in a humidified 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% (v/v) FBS (Life Technologies Corp), 100 U/mL penicillin and 100 mg/mL streptomycin (Sigma-Aldrich, St. Louis,

MO). For MTT assays, cells were seeded 16-24 hours before experiments in clear flat-bottom 96-well culture trays (Corning Life Sciences, Corning, NY) at a density of 3.0×10^4 cells/well.

A master 5 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was prepared using PBS for the MTT proliferation assay. After cell seeding, 20 µl of the master MTT solution was introduced with 180 µl of culture medium. Cells were incubated for 24 hrs after which 100 µl of dimethyl sulfoxide (DMSO) was introduced to dissolve the formazan crystals formed. Then, absorption at 570 nm was measured for each well while the absorption at 630 nm was used as reference.

1. Molina-Ontoria, A.; Chaur, M. N.; Plonska-Brzezinska, M. E.; Echegoyen, L., Preparation and characterization of soluble carbon nano-onions by covalent functionalization, employing a Na-K alloy. *Chem Commun (Camb)* **2013,** *49* (24), 2406-8.
2. Wajs, E.; Molina-Ontoria, A.; Nielsen, T. T.; Echegoyen, L.; Fragoso, A., Supramolecular solubilization of cyclodextrin-modified carbon nano-onions by host-guest interactions. *Langmuir* **2015,** *31* (1), 535-41.
3. Cunci, L.; Velez, C. A.; Perez, I.; Suleiman, A.; Larios, E.; Jose-Yacaman, M.; Watkins, J. J.; Cabrera, C. R., Platinum electrodeposition at unsupported electrochemically reduced nanographene oxide for enhanced ammonia oxidation. *ACS Appl Mater Interfaces* **2014,** *6* (3), 2137-45.
4. Cunci, L.; Vargas, M. M.; Cunci, R.; Gomez-Moreno, R.; Perez, I.; Baerga-Ortiz, A.; Gonzalez, C. I.; Cabrera, C. R., Real-Time Detection of Telomerase Activity in Cancer Cells using a Label-Free Electrochemical Impedimetric Biosensing Microchip. *RSC advances* **2014,** *4* (94), 52357-52365.