Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Photoluminescent Nano Graphitic/Nitrogen-doped Graphitic Hollow Shells as a Potential Candidate for Biological Applications

Supporting Information

Detailed Sample Preparation Methods

Magnetite (Fe_3O_4) nanoparticles (NPs) (1): Mono-dispersed magnetite NPs were prepared from an iron oleate precursor. In a typical synthesis, 40 mmol of iron chloride hexahydrate ($FeCl_3$•$6H_2O$, 98 %, Aldrich) and 120 mmol of sodium oleate (95%, TCI) were dissolved in a solvent mixture of 80 mL ethanol, 60 mL deionized water, and 140 mL hexane. This solution was heated to 70 °C and held for four hours. Immediately after the thermal treatment, the upper organic layer, containing the Fe-oleate complex, was removed and washed with water. For the formation of magnetite NPs, 40 mmol of dried iron oleate complex and 20 mmol of oleic acid (90 %, Aldrich) were dissolved in 200 g of 1-octadecene (90 %, Aldrich). The temperature was increased to 320 °C, and held for 30min. The resulting solution was slowly cooled to room temperature, and suspended black particles were precipitated by excess acetone and collected by centrifugation.

Magnetite@silica-encapsulated core-shell spheres (Fe_3O_4@SiO_2) (2): The silica-coated magnetite NPs were prepared by the water-in-oil micro-emulsion technique using tetraethyl orthosilicate (TEOS). For a typical synthesis, magnetite NPs (1) pre-dissolved in cyclohexane was injected into 8mL of the surfactant Igepal CO-520 (Aldrich) pre-dissolved in 200 mL of cyclohexane. In particular, we varied the amount of magnetite NPs dissolved in cyclohexane from 0.4 mg to 2 mg/mL, aiming for a silica-shell thickness of 7 nm to 12 nm. Next, 1.3 ml of 30 % NH_4OH solution (Aldrich) was added with stirring for several hours, and then 3 mL

of TEOS were added. The mixture was stirred for 12 hrs at room temperature. The resulting dark brown silica-coated NPs were washed with ethanol and centrifuged.

Silica@graphitic hollow spheres (SiO_2@GHS) (3) or magnetite@silica@graphitic hollow spheres (Fe_3O_4@SiO_2@GHS): Fe_3O_4@SiO_2 (2) pre-dispersed in an aqueous glucose solution was transferred into a Teflon lined stainless steel autoclave. The temperature was increased to 180 °C and maintained for 4 - 16 h. After the reaction went to completion, the reactor was rapidly cooled with cold tap water. The resulting solution was centrifuged at 6000 rpm for 30 min and the most upper, bright-yellow solution containing SiO_2@GHS NPs was separated. Deionized water was added to this solution and the mixture was centrifuged at 18000 rpm for 40 min. The dark brown suspension was separated and dried under a vacuum. To prepare a thermally annealed sample, the dried samples were heated at 450 °C in Ar atmosphere.

Graphitic hollow spheres (GHS) (4) or magnetite encapsulated graphitic hollow spheres (Fe_3O_4@GHS): The dried SiO_2@GHS (3) or Fe_3O_4@SiO_2@GHS were transferred to a polyethylene bath of 7 M HF solution and stored to allow chemical etching to remove undesired impurities. A yellowish-brown powder floating on the surface of the HF solution was obtained and washed with water, which included high-speed centrifugation. The resulting NPs were dried at 100 °C in a convection oven overnight. In addition, to make the thermal annealed sample, the dried NPs were heated in Ar atmosphere at 450 °C for one hour.

Nitrogen-doped graphitic hollow spheres (N-GHS) (5): N-GHS solution was achieved using ammonia and triethylamine (TEA) as reducing agents and nitrogen sources under a hydrothermal environment [10]. 30 % ammonia was added to 70 mL of the described GHS (4) solution to adjust the pH value to 10. Then 1mL of TEA was added. The solution was

then transferred into a Teflon-lined autoclave and heated at 80 °C for 3 h. The N-GHS were collected with centrifugation, followed by washing with deionized water several times.

Detailed Cell Culture and Cytotoxicity Experimental Conditions

The human cervical adenocarcinoma HeLa cells (2×10^4 cells) grown on coverslips were incubated with 200 μg/mL nanoparticles for 2 h. The cells were washed several times with ice-cold phosphate-buffered saline (PBS), fixed by incubation with freshly made 3% formaldehyde (containing 1.5% methanol) in PBS (pH 7.4) for 15 minutes, and neutralized with 50 mM NH_4Cl solution. After washing, the cells were visualized by fluorescence microscopy using an Axioplan Zeiss microscope and photographed with a digital camera (CCD). For Laser Scanning Confocal Microscopy (LSCM), the cells were visualized using a Zeiss LSM 510 confocal microscope (Nikon, Japan) equipped with Argon (488 nm) and HeNe (543 nm) lasers for fluorescence.

Cytotoxicity was assessed by measuring effects of GHS on cell proliferation and membrane damage, respectively. Cells (2 X 103 cells/100 μL) were seeded onto 96-well plates and incubated overnight at 37 °C under a 5 % CO2 atmosphere. The medium in the wells was then replaced with fresh medium containing GHS (1 ~ 1000 μg/ml) and incubation continued for 72 h. The effect of the GHS on cell proliferation was determined by WST-1 assay (Roche). Cells (2×10^3 cells/100 μl) were seeded onto 96-well plates and incubated overnight at 37°C under a 5 % CO_2 atmosphere. The medium in the wells was then replaced with fresh medium containing GHS (1-1000 μg/ml) and incubation continued for 72 h. Briefly, 10 μL of WST-1 solution (Roche) was added to each well and the plates were further incubated. After 4 h, the absorbance was measured with a plate reader at 440 nm. Cells incubated without GHS were used as a control. The release of lactate dehydrogenase (LDH) was monitored with

the CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega). Cells (2 X 104 cells/ml) grown on 24-well plates were incubated with GHS (0.5 ~ 1000 μg/mL) for 72 h. The plates were centrifuged, and aliquots (50 μL) of cell culture medium were collected from each well and placed in new microtiter plates. Finally, 50 μL of substrate solution was added to each well and the plates were further incubated for 30 min at room temperature. The absorbance at 490 nm was measured with a microplate reader. Cytotoxicity is expressed relative to basal LDH release by untreated control cells.

Fluorescence quantum yield measurement

For fluorescence quantum yield measurement of the GHS, we employed a comparative method [1, 2, 3, 4, 5] that relies on the use of fluorescence standards-organic fluorophores with known fluorescence quantum yields. In the same instrument, the unknown quantum yield may be calculated using the following equation:

$$QY_x = QY_{st}\left[\frac{A_{st}}{F_{st}}\frac{F_x}{A_x}\frac{n^2}{n^2{}_o}\right] = QY_{st}\left[\frac{G_{rad_x}}{G_{rad_{st}}}\frac{n^2}{n^2{}_o}\right]$$

where QY is the quantum yield, A is absorbance at the excitation wavelength, F is the integrated emission area, n and n_0 are refractive indexes of the solvent containing the unknown and the standard, x and st stand for a sample with unknown quantum yield and the standard. *Grad x* and *Grad st* are gradients obtained from the plot of the integrated fluorescence intensity vs. optical density.

Graphite hollow spheres were dispersed in water. The reference compound, L-Tryptophan (QY 14 %) [2,5], was dissolved in a phosphate buffer (pH=7.2). The difference in the refractive indices for water and buffer solution was within 1%. Concentration series were run on all the samples and the standard so that the absorbance values fell between 0.01 and 0.1

(for 10 mm path length fluorescence cuvette) at the excitation wavelength (285 nm). PL measurements of samples and the reference were conducted under identical conditions of excitation wavelength (285 nm) and slit settings at room temperature. The area under the PL curve in the range 300-550 nm, its integrated intensity, was calculated for each sample. We compared this result with results from other carbonaceous material, namely expanded graphite [6,7], which demonstrated a QY of ~0.10% and 0.08% for carbon onions [1], and 0.8% for oxidized carbon NPs from candle soot [8]. For water suspensions of isolated carbon nanotubes encapsulated in micelle-like cases of surfactant, the following QYs were observed: 0.01 % [9], 0.017 % [10], 0.1 % [11] and 1.1 % [3].

Figures

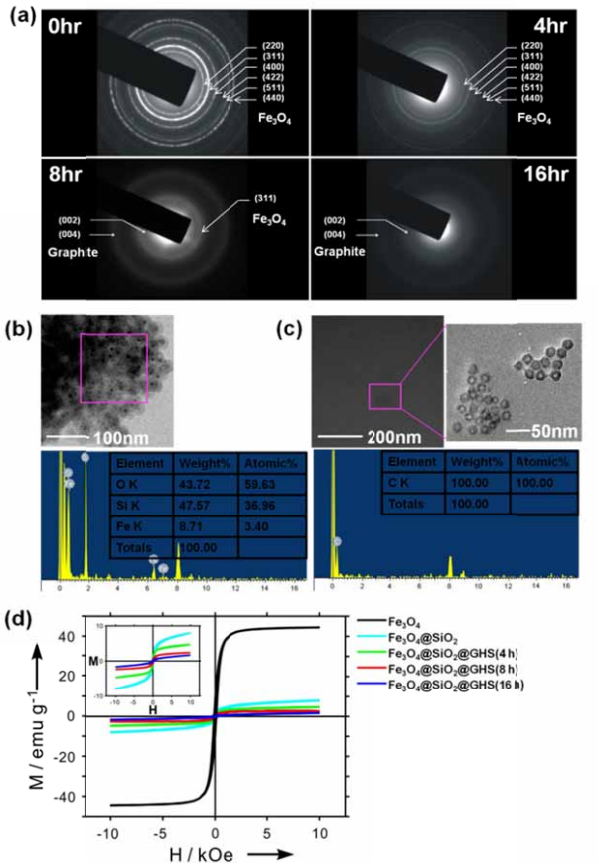

Figure S1. (a) Time-dependant void formation in Fe_3O_4@SiO_2@GHS during hydrothermal graphite deposition reaction from glucose on Fe_3O_4@SiO_2 (2), as monitored by using SAED and corresponding crystal planes labeled in each image. EDX results of (b) Fe_3O_4@SiO_2 (2) and (c) GHS (4) by HRTEM. (d) The SQUID magnetic measurement results, M-H loops at 5 K for Fe_3O_4 (1), Fe_3O_4@SiO_2 (2), Fe_3O_4@SiO_2@GHS 4h, 8h and 16 h samples. The saturated magnetization (Ms) data at 300 K for Fe_3O_4 (1) (44.0 emu g^{-1}), Fe_3O_4@SiO_2 (2) (8.1 emu g^{-1}) and this value decrease by reaction time increased, indicating that Fe_3O_4 core dissolved by reaction.

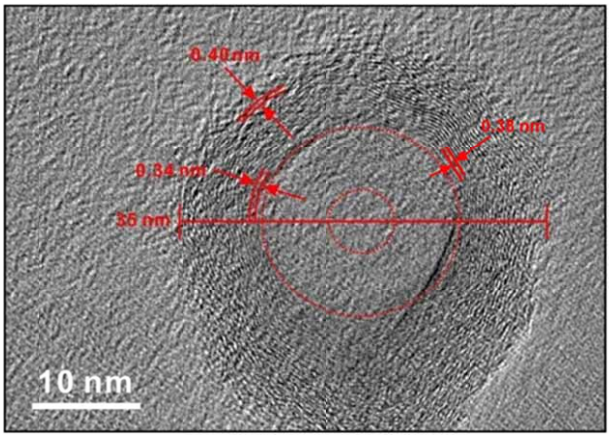

Figure S2. HRTEM image of final resultant, GHS (4).

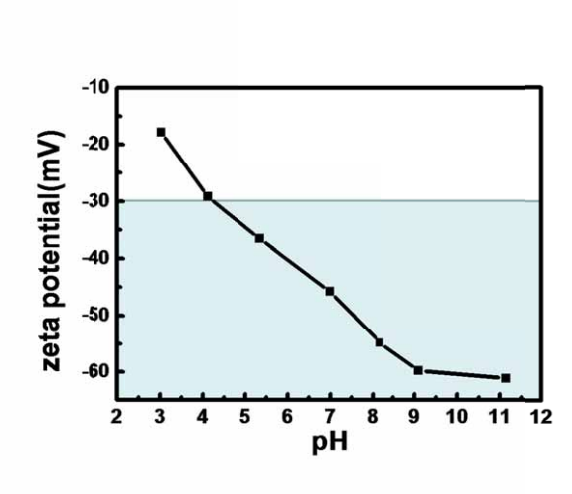

Figure S3. Zeta potential of GHS as a function of pH, in aqueous dispersions.

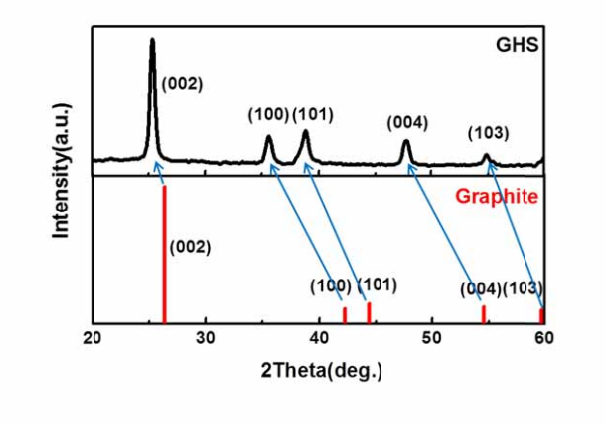

Figure S4. X-ray diffraction (XRD) patterns for GHS (**4**) annealed at 450°C in Ar and calculated XRD patterns of graphite (JCPDF #411487, a = 2.470, c = 6.724).

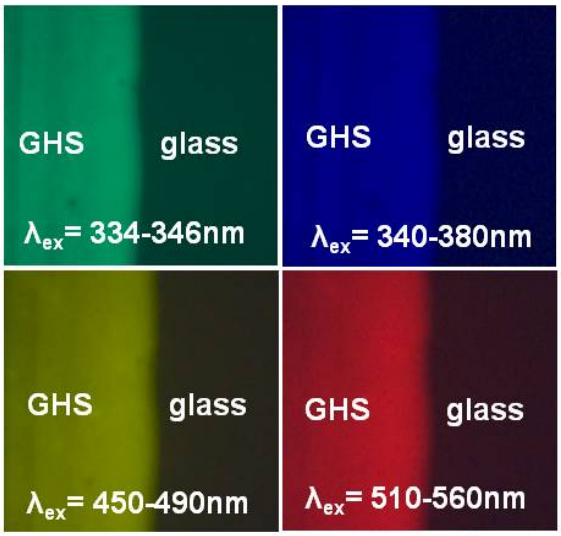

Figure S5. Fluorescent microscopy images of GHS on glass substrate with an excitation wavelength of 334 - 346 nm, 340 - 380 nm, 450 - 490 nm, and 510 - 560 nm.

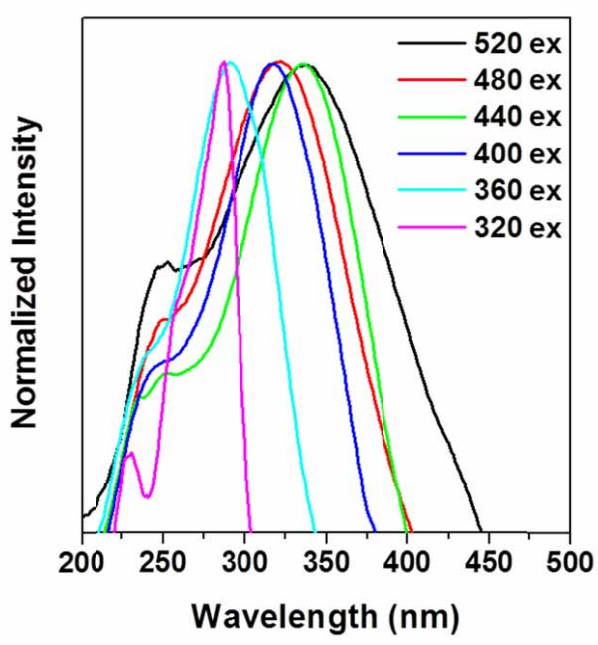

Figure S6. Excitation spectra of GHS, each spectra normalized to the same maximum intensity.

References for Supporting Information

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