Electronic Supplementary Information

Facile, Green Synthesis of Highly Fluorescent Carbon Nanoparticles from Oatmeal for Cell Imaging †

Caiyan Yu, Tongtong Xuan, Yiwei Chen, Zhenjie Zhao, Zhuo Sun, Huili Li*

Engineering Research Center for Nanophotonics& Advanced Instrument, Ministry of Education, Department of Physics, East China Normal University, Shanghai 200062, China.

***Corresponding author:* Prof. Huili Li**

E-mail:*hlli@phy.ecnu.edu.cn*;

Tel:* +86-21-62235465*;

***Fax:* +86-21-62234321.**

Experimental Section

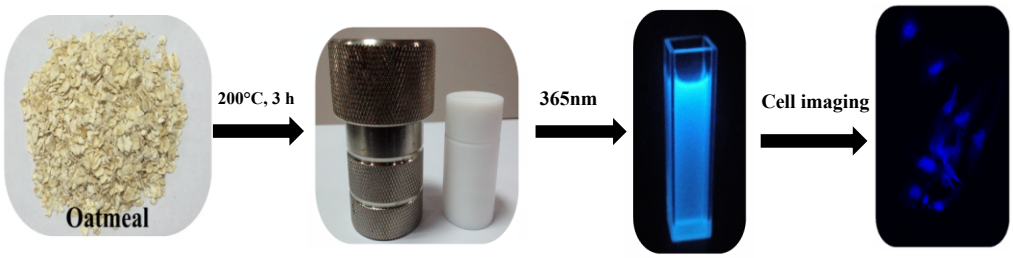
1. Reagents

The oatmeal (food grade, Seamild, Australia) was purchased from an online supermarket named JD.Com and triturated by ball mill for further use. Quinine sulfate and H_2SO_4 (98%) were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Dialysis membranes of 500-1000 Da (USA, Spectrumlabs) were purchased from Toscience Biotechnology Co, Ltd. (Shanghai, China). Cell Counting Kit-8 (CCK-8) and Bovine serum albumin (BSA) were purchased from Sigma-Aldrich Trading Co, Ltd. (Shanghai, China). Fetal bovine serum (FBS), trypsin and Dulbecco's modified Eagle's medium (DMEM) were obtained from Gibco Life Technologies Co. (Grand Island, USA). Human cervical carcinoma (HeLa cells) was purchased from Shanghai Institute of Cell Biology (Shanghai, China). All other chemicals were analytical grade and used as received without further purification. Distilled water was used throughout the whole experiment.

2. Synthesis of CNPs

0.1~0.7g triturated oatmeal powder was dispersed into 6 mL distilled water under vigorous stirring. Subsequently, the above mixture solution was added into a 10

mL Teflon-lined stainless steel autoclave and heated at 160~220°C for 1~7 h. The dark brown products were obtained after cooling to room temperature. The large and agglomerated nanoparticles were removed by centrifugation at 12,000 rpm for 15 min. The resultant supernatant containing CNPs was filtered through a 0.22 μm microporous membrane to further remove residual large particles. Final purification of the CNPs solution was conducted through a dialysis membrane (MWCO: 500-1000 Da) for 48 h (changed deionized water every 4 h). After this process, a light brown CNPs aqueous dispersion was obtained for further characterization. Scheme S1 illustrates the formation of CNPs from oatmeal through a simple hydrothermal method.



Scheme S1 Illustration of the formation of CNPs by the hydrothermal treatment of oatmeal.

3. Cell toxicity test

Cell Counting Kit-8 (CCK-8) assay was carried out to evaluate the cytotoxicity of as-prepared CNPs on HeLa cells (human cervical carcinoma cells) according to the following procedure. Briefly, HeLa cells were seeded in 96-well plates at 1×10^4 cells per well in Dulbecco's Modified Eagle's Medium (DMEM) medium with 10% fetal bovine serum (FBS) and incubated at 37°C in a humidified atmosphere with 5% CO_2. After incubating the cells for 24 h, the medium was replaced with 100 μL of fresh medium containing the different concentration of CNPs (from 0 mg mL^{-1} to 2 mg mL^{-1}). At certain times (24 h or 48 h), the medium was removed, and fresh medium (100 μL) containing CCK-8 (20 μL, 5 mg mL^{-1}) was added into each well, and then the cells were incubated at 37°C for another 4 h. The relative viability of cells was assessed by measuring the absorbance of the solution at 450 nm using a microplate reader (Multiskan MK3, Thermo).

4. Cell imaging

HeLa cells (1×10^4) were plated in a cover-glass-bottom dish in DMEM supplemented with 10% FBS. After culturing overnight in a 5% CO_2 humidified incubator at 37 °C, the culture medium was replaced with fresh DMEM (supplemented with 10% FBS) containing CNPs of 1 mg mL^{-1}. And then, the cells were further incubated for 2 h and rinsed with phosphate buffered saline (PBS) for three times to remove the excess CNPs. Finally, the images were taken by a confocal laser scanning microscope (Carl Zeiss LSM 700) excitated at 360 nm.

5. Characterization

The phase evolution was characterized by a M21XVHF2Z (Mac Science Co. Ltd.) X-ray diffractometer (XRD), using Cu Kα radiation (λ= 1.5405Å) at a voltage of 40 kV and a current of 40 mA with 2θ scanning mode. The morphology and particle size of CNPs were observed by a JEM-2100 high-resolution transmission electron microscope (HRTEM, JEOL, Japan) at an operating voltage of 200 kV. Dynamic light scattering (DLS) measurements of particle size in distilled water and serum media were carried out using a BI-200SM multi-angle dynamic/static laser scattering instrument (Brookhaven, USA). Raman spectra were recorded on a Raman Spectrometer (RM-1000, Renishaw) with 632.8 nm He-Ne laser as an irradiation source. X-ray photoelectron spectroscopy (XPS) data were obtained by a Multifunction Imaging Photoelectron Spectrometer (Thermo ESCALAB 250XI) with monochromatic Al Kα radiation (1486.6eV). Fourier transform infrared spectra (FTIR) in the region from 4000 to 500 cm^{-1} were recorded on a Nicolet Nexus 600 FTIR spectroscope (Nicolet Instrument Co., USA). The absorption and photoluminescence (PL) spectra of the as-prepared CNPs were measured by a UV-Vis spectrophotometer (Hitachi U-3900) and a fluorescence spectrophotometer (Horiba Jobin Yvon, FluoroMax-4), respectively. The quantum yield (QY) of the CNPs was calculated by the following equation:

$$QY_x = QY_R \frac{I_x A_R n_x^2}{I_R A_x n_R^2} \qquad (1)$$

Where I is the measured integrated emission intensity, n is the refractive index of the solvent, and A is the absorbance at the excitation wavelength, which is measured on a UV-Vis spectrophotometer. The subscript ‘‘*x*’’ and “*R*” designate CNPs and quinine sulfate, respectively. Quinine sulfate in 0.1 M H_2SO_4 was chosen as a standard sample. QY_R = 54% at 360 nm and n_R = 1.33.[1-3] The CNPs were dissolved in distilled water with n_x = 1.33. In order to minimize the effect of reabsorption, the absorbance values of all samples at the excitation wavelength were kept below 0.10.

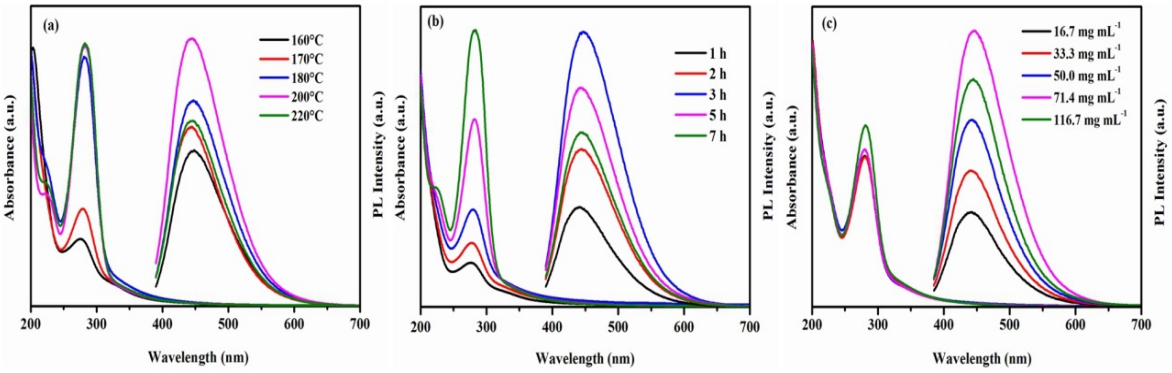


Fig. S1 The UV-Vis absorption and PL spectra (λ_{ex}=360 nm) of the CNPs prepared at different reaction temperatures (a) for 1~7 h (b) with various precursor concentrations (c).

Table S1 The atomic percentage of C and N elements and QY for oatmeal starting material and CNPs prepared at different temperatures.

Elements / Temperature	C/at %	N/at %	N/C/%	QY/%
Oatmeal	74.65	3.09	4.14	0
160°C	81.67	1.40	1.71	25.36
170°C	82.65	2.47	3.00	28.11
180°C	84.15	1.22	1.45	35.96
200°C	77.85	2.93	3.76	37.40
220°C	83.76	1.91	2.28	33.40

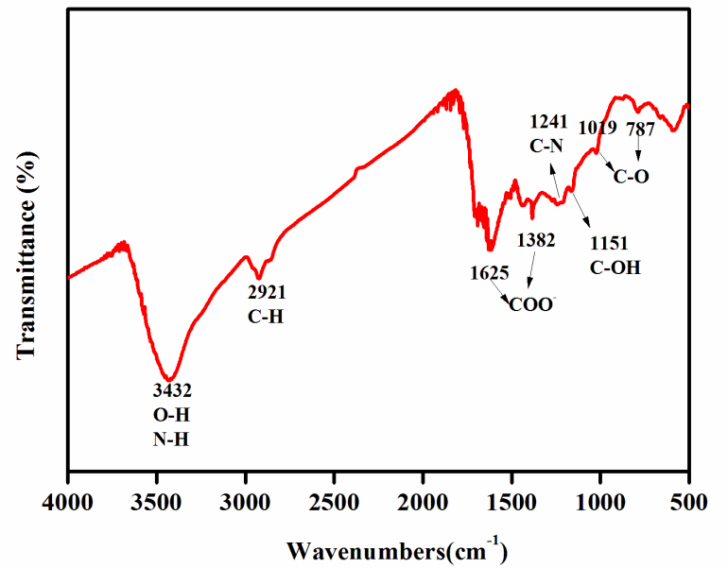


Fig. S2 FTIR spectrum of as-prepared CNPs.

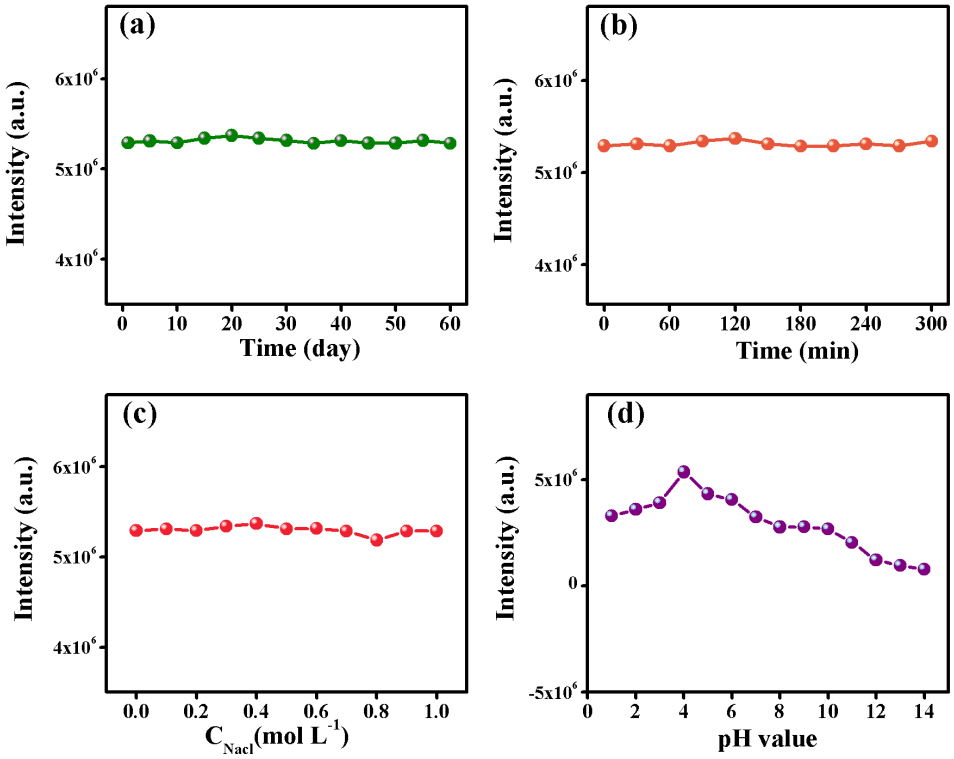


Fig. S3 Effects of storage time (a) and irradiation time (b) on PL intensity of CNPs in distilled water and effects of ionic strengths which are controlled by various concentrations of NaCl in aqueous solution (c) and pH value (d) on PL intensity of CNPs (λ_{ex} = 360 nm).

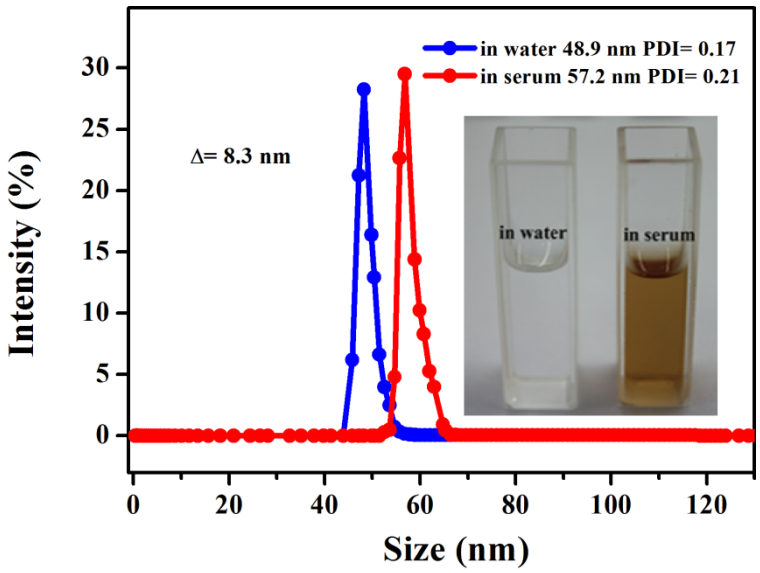


Fig. S4 Distribution plot of the hydrodynamic size of the CNPs in distilled water and serum media, respectively. The bottom right inset: photographs of the CNPs in distilled water and serum taken before DLS experiment.

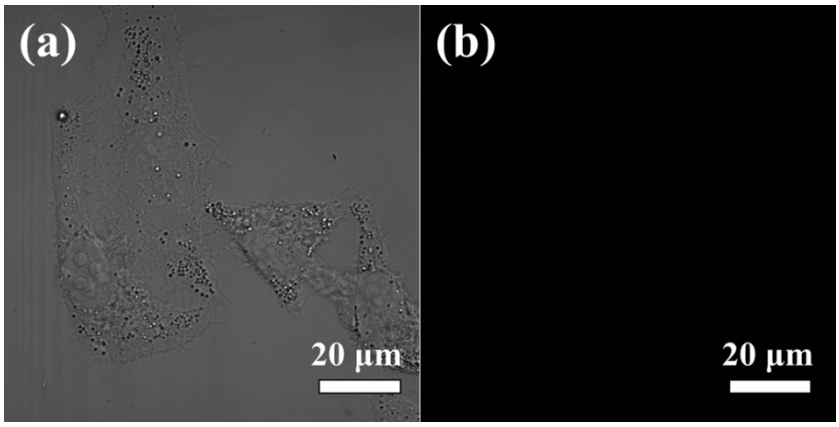


Fig. S5 Optical images of HeLa cells incubated in the absence of CNPs under bright field (a) and UV light (b). The dark field image was taken by a confocal laser scanning microscope (Carl Zeiss LSM 700) excitated at 360 nm. Cells were imaged using a 63× oil-immersion objective.

References

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