Supporting information

Surfactant-Free Synthesis of Graphene Oxide Coated Silver Nanoparticles for SERS Biosensing and Intracellular Drug Delivery

Fanyu Zeng,[†,#,‡] *Dandan Xu,*[†,#,‡] *Chen Zhan,*[†,#] *Chunyan Liang,*[†,#] *Weiwei Zhao,*[†,#,§] *Jiaheng Zhang,*[†,#] *Huanhuan Feng**[,†,#] *and Xing Ma**[,†,#,§]

[†] State Key Laboratory of Advanced Welding and Joining, Harbin Institute of Technology, Shenzhen 518055, China

[#] Research Centre of Printed Flexible Electronics, School of Materials Science and Engineering, Harbin Institute of Technology, Shenzhen 518055, China

[§] Key Laboratory of Micro-systems and Micro-structures Manufacturing of Ministry of Education, Harbin Institute of Technology, Harbin 150001, China

* E-mail addresses: maxing@hit.edu.cn, fenghuanhuan@hit.edu.cn.

‡ These authors contributed equally.

EXPERIMENTAL SECTION

Materials and Instruments. Graphite powder, (H_2SO_4, 36%), potassium permanganate ($KMnO_4$), hydrogen peroxide (H_2O_2, 30%), hydrochloric acid (HCl 37%), nitric acid (HNO_3 68%), silver nitrate ($AgNO_3$, 2.5%), sodium borohydride ($NaBH_4$), foetal bovine serum (FBS),

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), phosphate-buffered saline (PBS, pH 7.4) buffer, Trypsin-EDTA, 4',6-diamidino-2-phenylindole (DAPI), were purchased commercially,. SEM and TEM images were captured by FESEM S4700 at 15kV and Tecnai G2 Spirit at 120kV, respectively. UV-Vis absorbance spectra were obtained by a spectrometer (UV-2600, Shimadzu). Dynamic light scattering was carried out by Malvern Zetasizer Nano ZS90. Fluorescence emission spectra were obtained by an spectrofluorimeter (RF 5301, Thermo). Fluorescence images were captured by a Leica inverted optical microscope (Leica DMi8). The MTT assay was conducted by a microplate reader (F50, Tecan).

Preparation of NGO. 0.5 g graphite powder was added into 15 mL H_2SO_4 in an ice bath, then 1.5 g KMnO4 was added slowly under mild stirring. After the mixture was kept stirring at 50 ℃ for 3 hours, 35 mL of deionized (DI) water was added. After stirring for another15 minutes, 150 mL of deionized water was added to the above solution to terminate the reaction. When the mixed solution became bright yellow, 10 mL of H_2O_2 was added. The mixture was washed with HCl (10%) twice, then suspended into 200 mL HNO_3 (15%) was heated at 80 ℃ for 24 hours for further separation. Finally, the above mixture was washed with deionized water until the pH of the solution is neutral. The products were treated with sonication for 2 hours and then filtrated by 220 nm filter membrane to obtain NGO solution for further use.

Preparation of Ag@NGO. For a typical synthesis, the mixture of 10 mL $AgNO_3$ (0.02 M) and 10 mL NGO (0.5 mg/mL) solution was stirred for 20 min under an ice bath. Then, 10 mL $NaBH_4$ (0.02 M) solution was added into the above solution drop by drop until the mixture became yellow. The products were washed five times with deionized (DI) water to obtain Ag@NGO.

Preparation of Ag@NGO@DOX. Then, the mixture of 1mL (1mg/mL) Ag@NGO and 1mL (1 mg/mL) DOX was prepared first. The mixture solution was shaken for 10 minutes to facilitate the loading of DOX molecules. Then, the above mixture was centrifuged twice to remove the supernatant to obtain the drug loaded nanoparticle, denoted as Ag@NGO@DOX.

Cell culture. HepG-2 liver cancer cells were cultured in a complete cell culture medium containing of 89% DMEM, 10% FBS and 1% antibiotics. The cell culture was maintained at 37 $^{\circ}$C in a humidified atmosphere containing 5% CO_2.

Raman spectroscopy. Crystal violet was used as a model molecule to test the SERS properties of Ag@NGO. After 2 minutes of ultrasound, the mixture was injected into the capillary glass tube with a liquid transfer gun. The Raman spectra were measured by a 514 nm laser under the Raman spectrometer (Renishaw invia). The Raman spectroscopy of DOX was measured the same as that of crystal violet, and the measuring equipment is 633 nm laser (Invia basic). For intracellular SERS

measurement, the HepG-2 cells were co-cultured with Ag@NGO on the glass slide in the culture medium for 24 hours. The SERS spectra were measured by Raman spectrometer after washing the cells with PBS.

MTT cytotoxicity assays. The cell viabilities of Ag@NGO, DOX and Ag@NGO@DOX were evaluated by the MTT assay using HepG-2 cell line. 100 μL of HepG-2 cell suspension (1000 cells) was added to each well of a 96-well plate and cultured for 24 hours at 37 ℃ in 5% CO_2 atmosphere. 10 μL Ag@NGO, DOX and Ag@NGO@DOX solution were added into each well with different concentrations of 0, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 $\mu g \cdot mL^{-1}$. After co-incubation of samples and HepG-2 cells for 24 hours under the same conditions, the cell medium in each well was replaced by a new medium containing 90% culture medium and 10% MTT solution (0.5 mg/mL). After the plate was cultured in dark at 37℃ for another 4 h incubation, the cell medium was removed and 100 μL DMSO solution was added into each well. The microplate reader (Tecan infinite F50) was used to measure the absorbance intensity at 490 nm. The cell viability was obtained by comparing to the group of control without any treatment (100 %). Statistics were obtained with 6 duplicates for each concentration (n=8).

Fluorescence microscopy image. 1 mL HepG-2 cells were cultured in complete DMEM medium for 24 hours. 50 μL Ag@NGO@DOX (12.5 μg/mL) was added into the cells and co-cultured for 24 hours. Then the culture medium was removed, the cells were washed with 2 mL PBS twice and fixed with 1 mL paraformaldehyde (4%)

PBS solution for 15 minutes. Then the paraformaldehyde solution was removed, and the cells was washed with PBS three times. We stained the cell nucleus by DAPI (0.1 $\mu g \cdot mL^{-1}$) solution for 15 min. The cells were washed with PBS five times before observed under fluorescence microscopy.

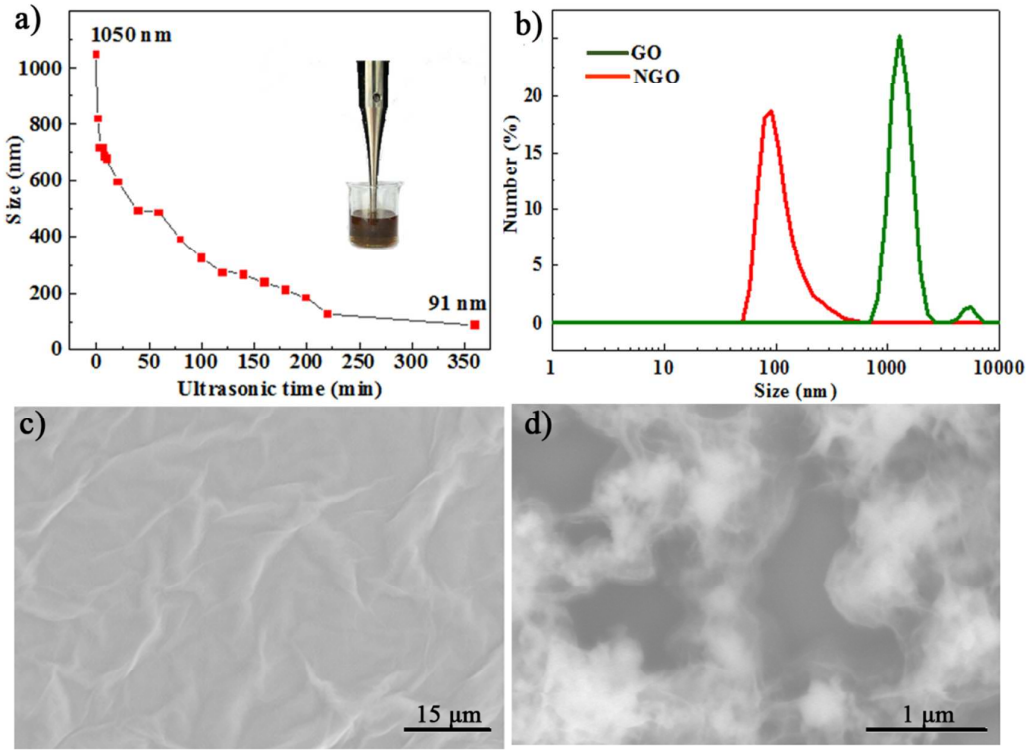

Figure S1. (a) Relation between GO size and ultrasonic treatment time (inset is the image of the setup of the probe sonication treatment); (b) Size distribution of GO and NGO; SEM image of (c) GO and (d) NGO.

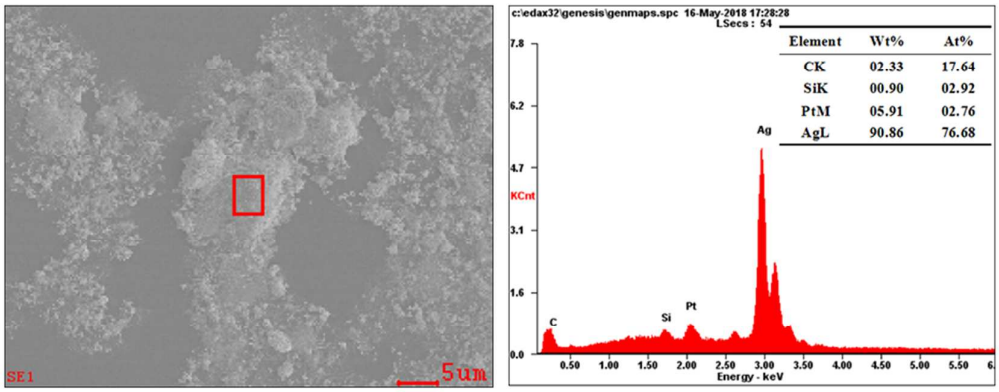

Figure S2. SEM image and EDS analysis of Ag@NGO.

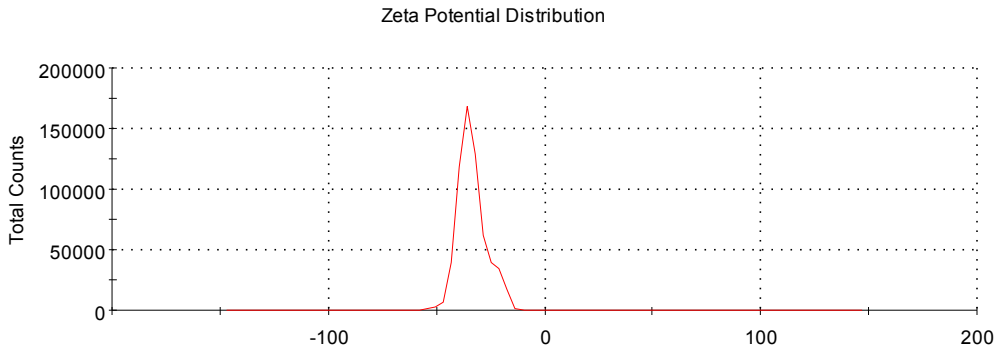

Figure S3. Zeta potential of Ag@NGO (-49.2 mV).

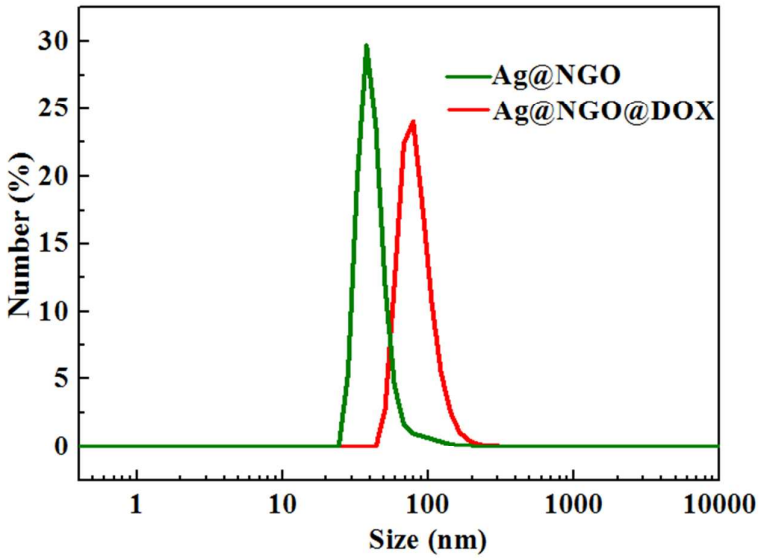

Figure S4. Size distribution of Ag@NGO and Ag@NGO@DOX in cell culture medium.

Discussion: We provided the DLS results of the Ag@NGO and Ag@NGO@DOX in cell culture medium containing 10% serum in Figure S4 in the SI. The particle size of Ag@NGO in culture medium is 42 nm, which is consistent with that in aqueous medium (Figure 1d). The particle size of Ag@NGO@DOX in culture medium is 84 nm, which is larger than that in DI water.

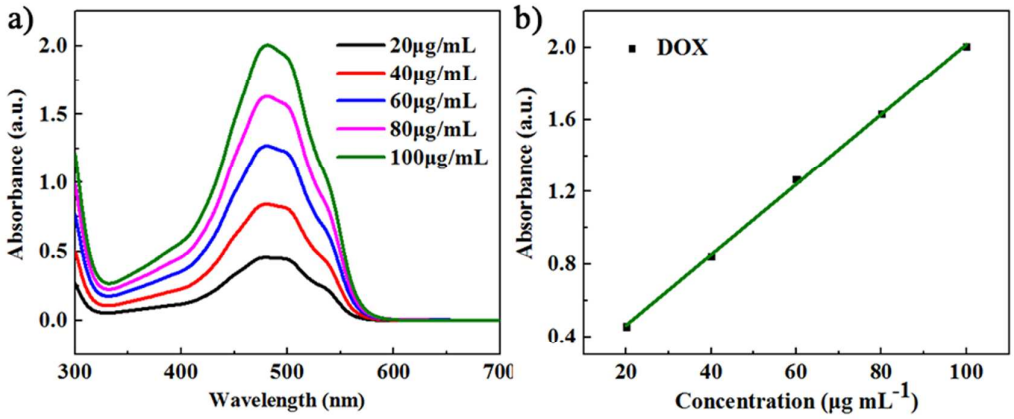

Figure S5. (a) UV-vis spectra of DOX with different concentrations, (b) Linear plot of the absorption intensity of DOX (480 nm) in the range of 20 μg/mL to 100 μg/mL.

Discussion: The DOX loading amount on the Ag@NGO nanoparticles were evaluated. The Ag@NGO@DOX nanoparticles were suspended in acidic solution to fully release the loaded DOX. Then, the UV-vis intensity of released DOX was measured and the DOX amount was quantified by using the standard curve of UV-vis absorbance intensity (480 nm). The DOX loading amount was found to be 8 wt% of the Ag@NGO nanoparticles.

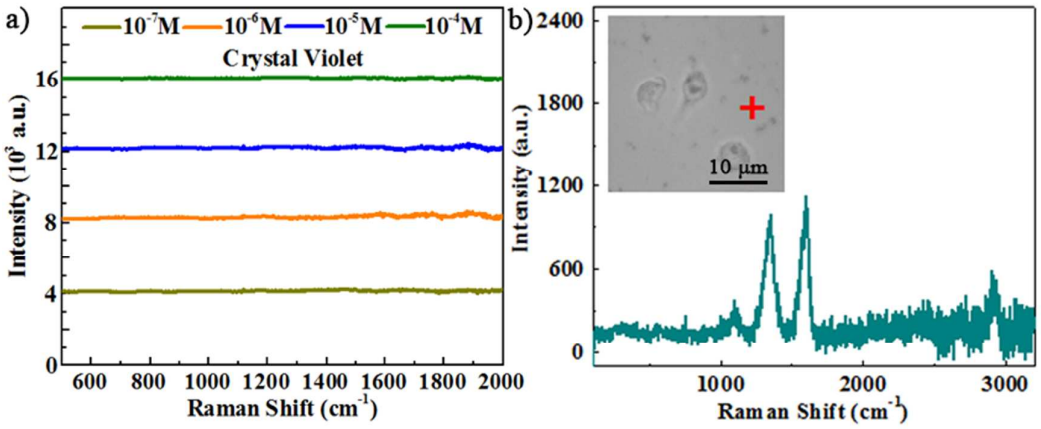

Figure S6. (a) Raman spectra of crystal violet different concentration range from 10^{-7} M to 10^{-4} M; (b) SERS spectra of Ag@NGO nanoparticles outside the cells.

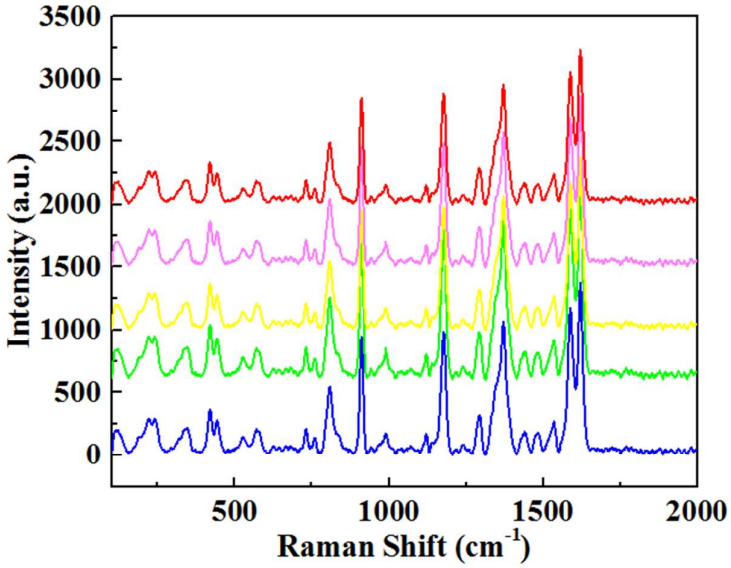

Figure S7. SERS spectra of 10^{-7} M crystal violet different repeat times.

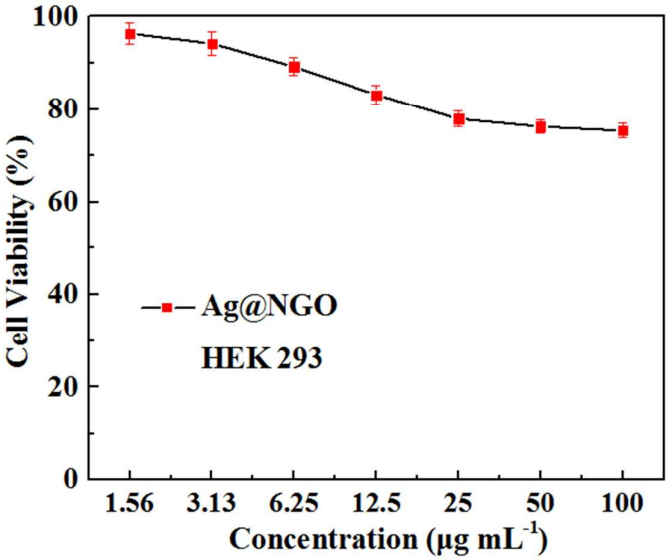

Figure S8. MTT cytotoxicity assay of HEK 293 cells after treated with Ag@NGO.