

Supporting Information

DOI: 10.1002/ ((please add manuscript number))

Quantitative study of intracellular concentration of graphene/noble metal nanoparticle composites and cytotoxicity

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Figure S1. Raman spectra of GO, GO/Au, and GO/Ag composites.

Raman spectra were recorded on a confocal Raman microscopy (inVia-Reflex, Renishaw) with 532 nm excitation. A drop of the sample suspension in water was added onto a glass slide, and dried at room temperature.

The presence of a broad G band at 1593 cm^{-1} and a D band at 1360 cm^{-1} was observed from GO sample. It is noteworthy that the intensity of the D band is comparable to that of the G band suggesting the presence of defects in the specimen. However, we did not observed clear 2D peak around 2700 cm^{-1} in the Raman spectra (data not shown). This might be attributed to the oxidation and thus reduced crystallinity of the original graphene sheets. ^[1-4]

The enhancement factors of GO/Au and GO/Ag were 1.83 and 4.70, respectively.

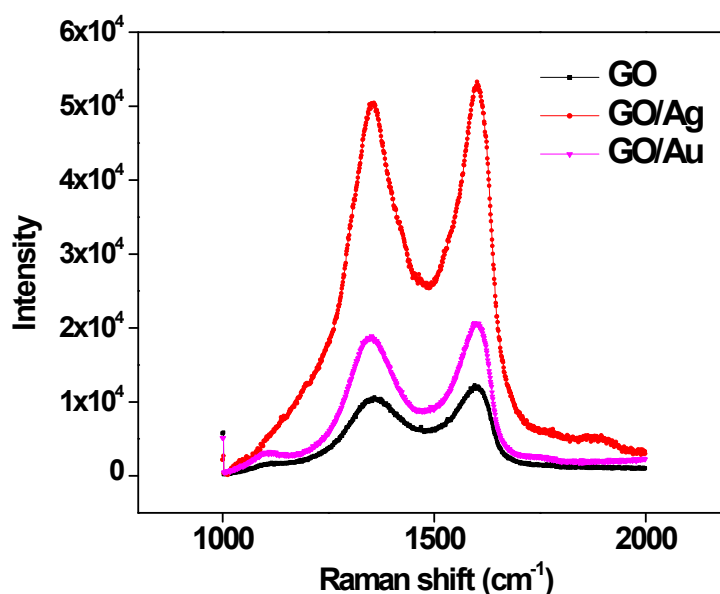
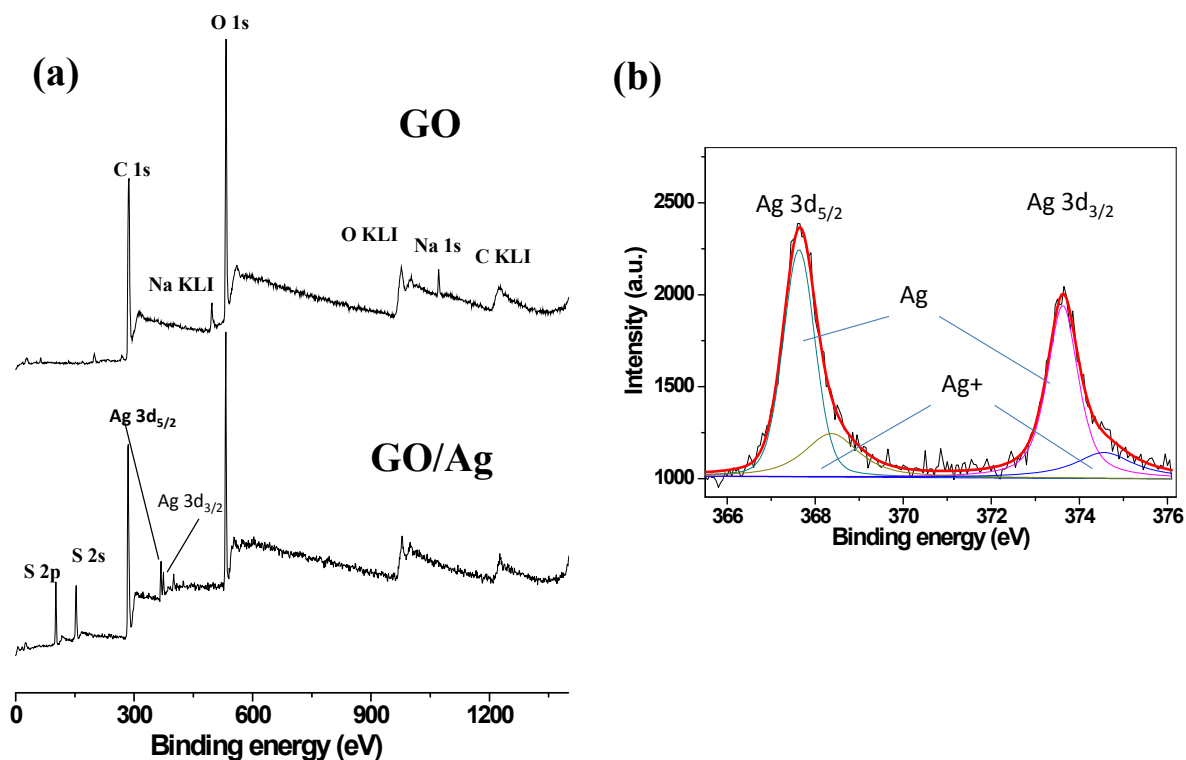


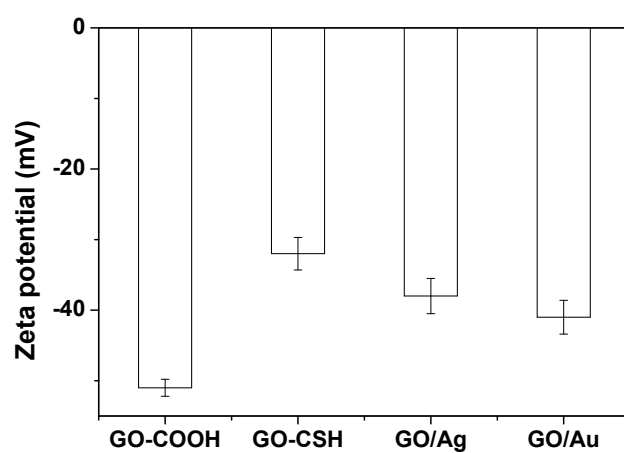
Figure S2 (a) XPS survey spectra of GO and GO/Ag composites. (b) High-resolution spectra of Ag 3d.



The chemical compositions of the surfaces were characterized by X-ray photoelectron spectroscopy (XPS) using an Axis Ultra spectrometer (Kratos Analytical, UK) with a monochromatized Al K α source at pass energy of 160 eV for survey scans and 80 eV for core level spectra. Data was analyzed by Kratos Vision Processing and XPS Peak software. The binding energy was corrected by setting the lowest binding energy of C 1s peak at 284.6 eV.

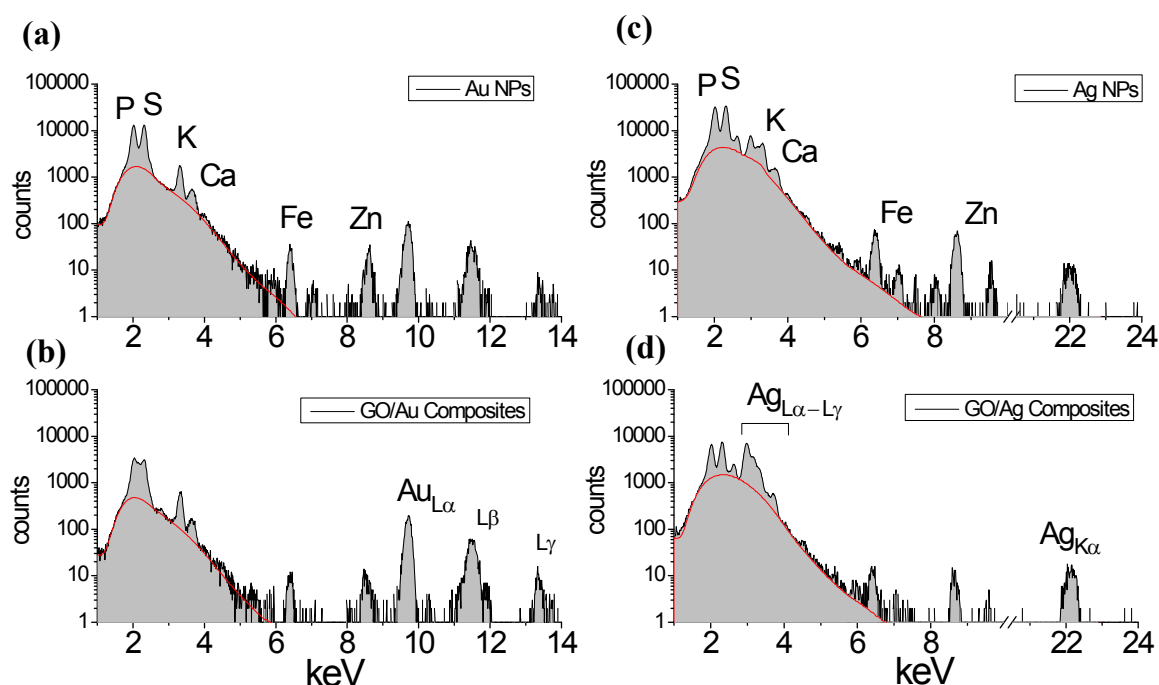
The results (Figure S2) showed that the C/O mass ratio is about 1.8, suggesting the C/O atomic ratio is about 2.4. The XPS spectra of Ag 3d_{5/2} show that there are two components in the GO/Ag composites, attributed to Ag⁺ (368.2 eV) and Ag⁰ (367.7 eV), respectively. ^[5] The atomic ratio of Ag⁰/ Ag⁺ is about 3.4, indicating over 20% of the silver element on the surface is ionized.

Figure S3 The surface zeta potential of GO and GO based composites.



The surface charge of GO based particles were determined using Beckman DelsaTM Nano (Beckman Coulter), with a He-Ne laser operating at a wavelength of 677 nm and room temperature. The particles (50 µg/mL) were dispersed in 10 mM NaCl.

Figure S4 Typical PIXE spectra of A549 cells treated with (a) Au NPs, (b) GO/Au composites, (c) Ag NPs, and (d) GO/Ag NPs, respectively. A549 cells were exposed to 5 $\mu\text{g/ml}$ metal NPs and 100 $\mu\text{g/ml}$ GO-based composites for 24 h, respectively. The red line represents the fit of the Bremstrahlung background.



The intracellular concentration was calculated by combination of parameters from PIXE, integral peak area, and RBS analysis, sample composition, thickness and accumulated charge, with the GeoPIXE II software^[1].

Each sample was scanned over 30 – 210 min, with a scan area of 625 – 2500 μm^2 . Beam-currents ranged between 200 and 800 pA. The particle induced X-ray emission (PIXE) detector (Canberra, Meriden, CT, U.S.A.) consists of a High Purity Germanium crystal (95 mm^2 active area) with an energy resolution of 148 eV at 5.9 keV. Additionally, the detector is covered with a 60 μm thick polyethylene window, in order to avoid penetration of backscattered protons. Backscattered protons were detected with a 300 mm^2 Canberra PIPS-detector (resolution 10.6 FWHM), covering a solid angle of 91.8 msr.

Both Au and Ag NPs are identified by the signal of the L-lines. Au showed three distinct L-lines between 9 and 14 keV, which were used for quantification (Figs. S4a,b). In case of Ag, an overlap between its L-lines and K-lines of K and Ca was observed in the PIXE spectrum between 2.5 and 4 keV. Therefore, the $K\alpha$ -line at 22 keV was used as a fingerprint of Ag (Figs. S4c,d). Nevertheless, deconvolution of PIXE spectrum and following calculation of Ag concentration were performed taking into consideration both the K and L lines.

Figure S5. The cells were exposed to 100 $\mu\text{g/ml}$ GO/Au and GO/Ag composites for 24 h. The backscattering signal images represent the distribution of carbon (top) for cells exposed to GO/Ag (left) and GO/Au (right). The co-localization of carbon with metal could be observed in those cells in which the cellular composite concentration was high enough to be visible over the cellular carbon background.

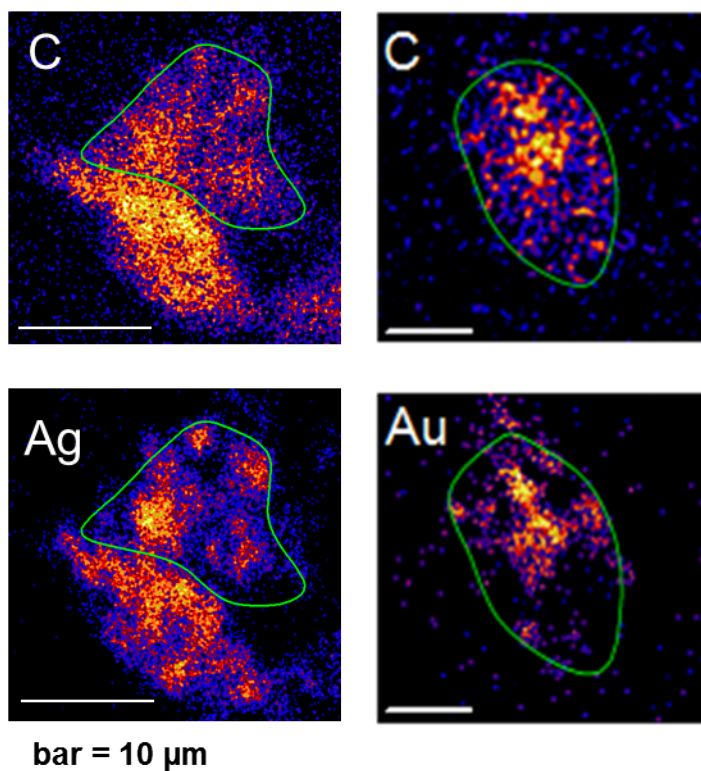


Figure S6 ICP-MS intracellular metal content of HepG2 cells after incubation with NPs and GO/NPs composites for 24h, respectively. * indicates significant difference at $p < 0.05$.

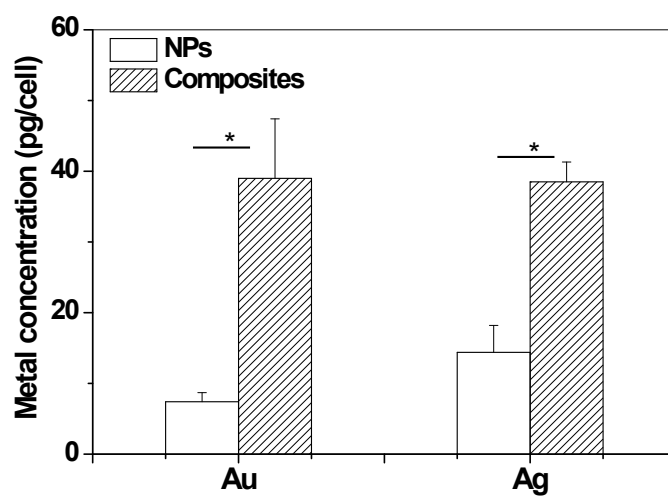


Figure S7. Relative (a) viability, (b) released LDH and (c) intracellular reactive oxygen species (ROS) of HepG2 cells incubated with 5 $\mu\text{g/mL}$ metal NPs and 100 $\mu\text{g/mL}$ GO/NPs composites with the same metal content for 24 h. The cells in particle-free culture medium were used as control. * indicates significantly difference at $p < 0.05$ level. (d) Relative cell viability of HepG2 cells incubated with metal NPs and GO/NPs composites for 24 h after pretreatment by NaN_3 (inhibits energy-dependent process) or under 4 $^\circ\text{C}$ for 1 h.

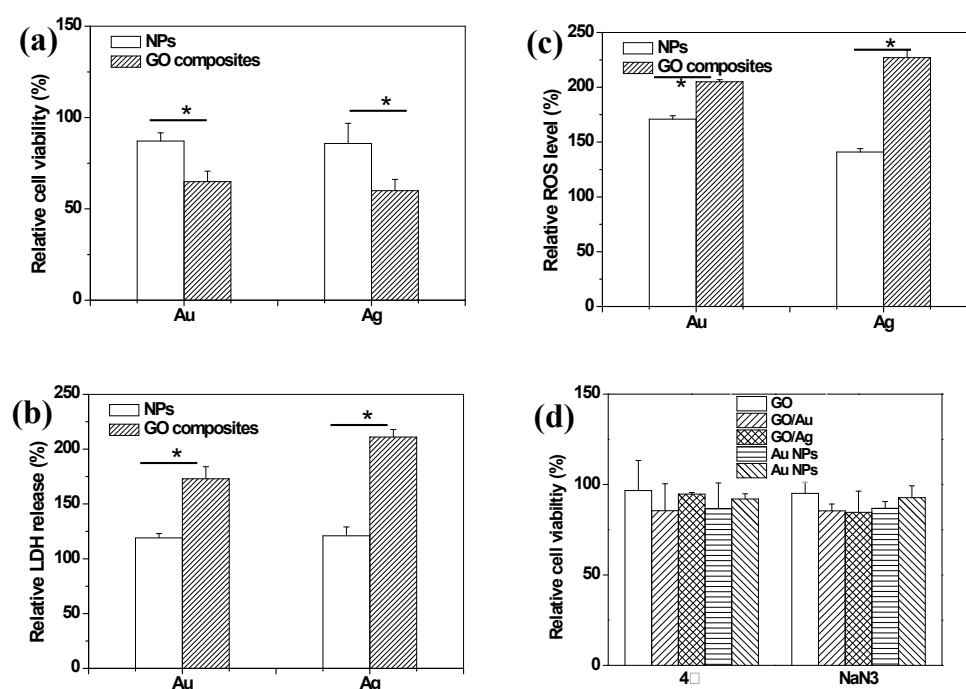
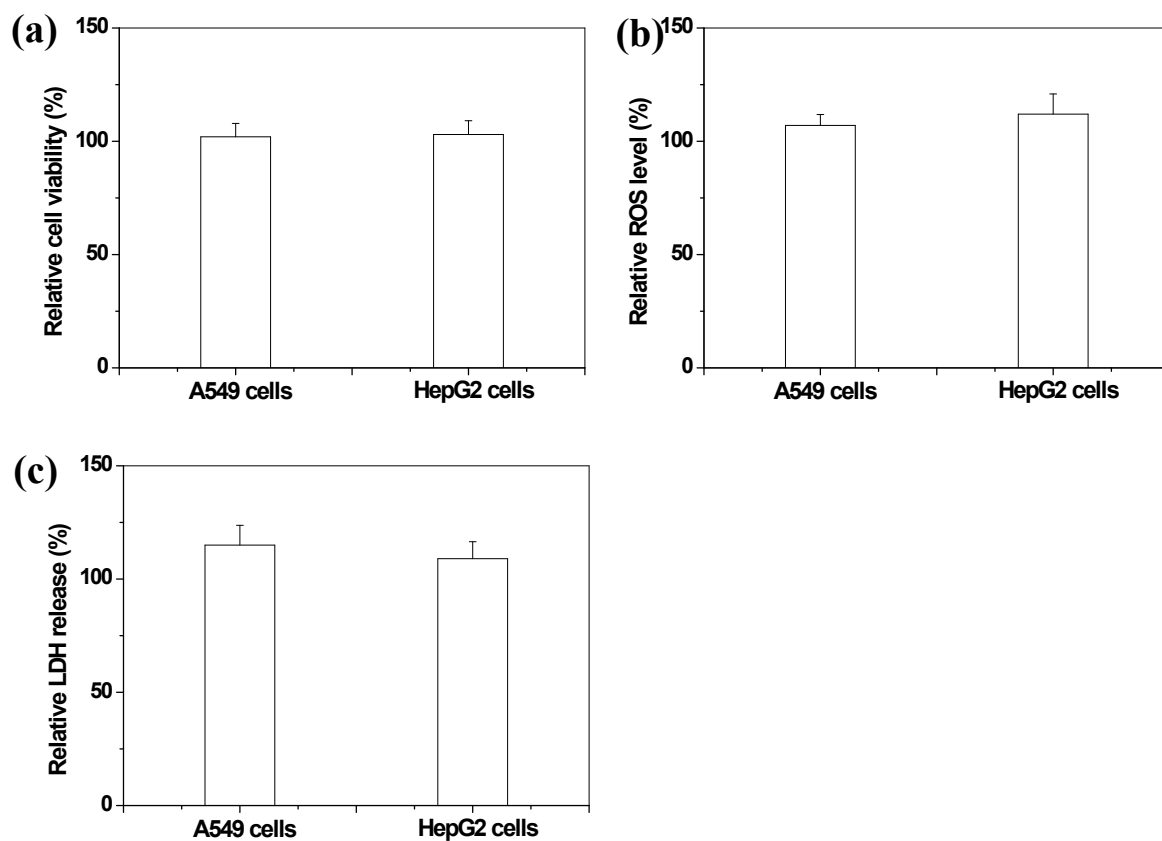


Figure S8. (a) Relative viability, (b) released LDH, and (c) intracellular reactive oxygen species (ROS) of A549 and HepG2 cells incubated with 100 $\mu\text{g/mL}$ GO particles for 24 h. The cells in particle-free culture medium were used as controls, respectively.



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